The Journal of Laboratory and Clinical Medicine

VOL. VI. ST. LOUIS, NOVEMBER, 1920 No. 2

ORIGINAL ARTICLES

HEMOSTATIC AGENTS AND THE SPONTANEOUS CHANGES IN COAGULATION TIME FOLLOWING HEMORRHAGE*

BY P. J. HANZLIK, M.D., CLEVELAND, OHIO

IN a recent number of this JOURNAL, (February, 1920, No. 5, p. 574) H. C. Hamilton, of Parke, Davis & Co., describes what he considers a superior method for testing hemostatic products, namely, observations on coagulation time of blood before and after intravenous injection. As he points out, the principle of this method has been tried by us in our work on the general subject of the comparative hemostatic efficiency of thromboplastic agents. The experiments that we had tried according to this principle, and which we described briefly in that paper, led us to the conclusion that this principle is not usable.

Hamilton considers that our results were unusual and exceptional, and thinks that they can only be explained by large hemorrhage, which he assumed to have occurred. In this he was mistaken. The quantities of blood that were withdrawn were about 4 c.c. at a time.

In view of this misunderstanding, it is well to give further details that were omitted from our original paper, because we considered them sufficiently implied in the data given. Dogs were used. These were previously morphinized, then etherized, placed on an operating table, and connected from the left carotid artery to an ordinary mercury manometer for recording changes in blood pressure, which was observed as the hemorrhages were continued, and subsequently during the injection of peptone. An ordinary glass cannula was tied in the left femoral artery for making the interrupted hemorrhages. A small serrefine clip was placed on the vessel central to the cannula. When the hemorrhages were made, the clip was loosened and 1 to 2 c.c.

*From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio.

of blood were allowed to escape into small vials of 4.5 cm. length and 1.2 cm. in diameter and with straight sides. Duplicate tests were made requiring the withdrawal of about 4 c.c. of blood at most into the vials plus the small quantity of blood left behind in the cannula. The vials were placed into a rack and allowed to stand at room temperature without further disturbance except for a slight and gentle tilting every 15 seconds to see whether the blood still flowed. If a movement of the meniscus was discerned the vials were replaced, but, if this was absent, the vials were completely inverted. Complete invertability without loss of contents constituted the endpoint of coagulation. In several instances it was possible to invert the vials without loss of contents almost immediately after completion of the hemorrhage, or in about 10 seconds, which was the shortest time in which all movements used in the procedure could be executed. At times the rapidity of clotting was indeed remarkable. We did not record the exact or even approximate percentages of loss of blood under the conditions of our experiment. What this was we do not know, but the absolute quantity of blood withdrawn at a given moment was small.

The results of the four experiments (see Table 5, page 205, paper by Hanzlik and Weidenthal; *Jour. Pharm. Exper. Therap.*, 1919, xiv, 189) performed by us showed the coagulation time to be invariably and markedly shortened as the hemorrhage progressed. In two of the animals, after the interrupted hemorrhages had been continued for some time, the coagulation time was somewhat prolonged, though it was still shorter than the coagulation time accompanying the first bleedings. Obviously, such results can inspire no confidence in the use of the method. Therefore, we still feel justified in suspending our work at this point, and in this we are sustained by some experiences of Professor Howell, who states as follows on page 297 of his Harvey Lecture for 1916-17 concerning his results with the intravenous and other methods of administering cephalin to dogs: "I have made a large number of experiments on dogs in which cephalin in aqueous solution was injected intravenously, intraperitoneally or subcutaneously or finally was fed by mouth. The results of these experiments have not been published since they showed *many irregularities* [italics mine] that will require further experimentation to explain."

Hamilton cites the experiments of Drinker and Drinker (*Amer. Jour. Physiol.*, 1915, xxxvi, 305, and not 1915, xxxviii, 233 as given by Hamilton) quoted by Howell, whom we quoted correctly though credited erroneously, that hemorrhage itself shortens coagulation time. Hamilton interprets the loss of blood during the hemorrhages in the experiments of Drinker and Drinker as "an approximate loss of 20 per cent of blood at one time, at another 33 per cent," but I am at a loss to find any such calculations in their paper, nor was it the object of these authors to study especially the relation of quantity of blood lost to the shortening of coagulation time. Moreover, the experiments of Drinker and Drinker were made on cats and rabbits, while our experiments were made on dogs, which precludes the possibility of making strict comparisons. It is a familiar and old observation that progressive bleeding shortens the coagulation time of blood. For instance, Gray and Lunt (*Amer. Jour. Physiol.*, 1914, xxxiv, 332) cite no less than 12 investi-

gators who have been concerned with this problem under various conditions, including very small capillary hemorrhages as from finger puncture to very large hemorrhages in animals and those accompanying surgical operations on human individuals. Whatever the cause of this phenomenon may be, it is obviously a rather variable function, and to make use of a method in which this occurs and propose it as a method of bio-assay for thromboplastic agents as Hamilton does is attempting more than we would be justified in doing.

On the contrary, Hamilton's results seem to us exceptional and to require further explanations. Two of these occur to us: (1) Selection of data and (2) manipulation of the clot. Hamilton apparently obtains his results by a process of selection which appears to us as questionable, for he states as follows on page 578 of his paper: "Animals have been rejected for testing purposes: (1) Because of having too short a coagulation time: it should not be less than 6 minutes. (2) Because of some unrecognized factors which influence the coagulation time before the agent is administered. A shortened coagulation time on the second sample of blood to one-third that of the first sample was once observed when not more than 10 c.c. had been withdrawn from a 12 kgm. dog. (3) Because of failing to react to an active sample of coagulant, whose potency had been demonstrated on 2 test animals." I doubt the ultimate value of conclusions obtained by rejecting systematically any results which do not fit into a preconceived scheme.

Undue disturbance of the blood in the vials prolongs the clotting time and may even inhibit the process of coagulation. Hamilton says on page 577 of his paper in this Journal, 1920, v, 574: "One must be careful that an apparently firm clot is not due to a skin over the surface below which the blood is fluid," an inference by which he apparently justifies the following interference with the clotting process as indicated in a previous paper by him (see page 122, *Jour. Am. Pharm. Assn.*, 1920, ix, 118) when he advises that "This should be broken so that correct observations can be made."

Until these criticisms are more satisfactorily explained we consider ourselves justified in having stopped further work with this method and we adhere to our former view, that the method employed by Hamilton, namely, testing of hemostatics on coagulation time before and after intravenous injection of these, does not justify conclusions as to comparative hemostatic efficiency of thromboplastic agents; and that the methods selected by us did justify us in concluding that hemostatic serum is inert as an accelerator of coagulation of blood and plasma *in vitro*, and as a hemostatic *in vivo*.

A PHARMACOLOGICAL STUDY OF BENZYL BENZOATE*

By Edward C. Mason, A.B., M.D., and Carl E. Pieck, B.S.,
Cincinnati, Ohio

THE recent introduction of benzyl benzoate to the Medical profession, and the apparently increasing demand for the substance have led us to undertake the present study in the hope of adding some further contribution to our knowledge of the pharmacologic action of the body. With reference to this type of benzyl derivatives it has already been stated by Macht that "inasmuch as they are practically insoluble in water, pharmacologic experiments with them could not be performed on isolated tissue *in vitro* except under special conditions." We have, of course, been confronted with this difficulty in carrying out the present experiments which have been performed entirely on the intact animal, since Macht,[1] in his splendid work on this subject, has probably done all that would yield valuable information through working with isolated tissues.

Three different preparations were used in the course of this work. They were sold by the following firms: Hynson, Westcott and Dunning, Baltimore; The Norwich Pharmacal Co., Norwich, New York; and Van Dyke and Co., New York. These three preparations varied somewhat in toxicity, and considerably in their property of remaining in solution. However, the pharmacologic effects produced were quite the same. By testing the action on drawn blood we found that these commercial 20 per cent preparations exercised a pronounced action on the drawn blood, and for that reason we have used the 20 per cent preparations diluted with an equal volume of water. We found this 10 per cent dilution very satisfactory for intravenous injection. In many of our records the term "saturated water emulsion" is used and is not technically correct, but refers to the 10 per cent solution just described.

In the recent medical literature[2] a considerable number and variety of clinical conditions are described as having been benefited, often in a very striking manner, by the use of the benzyl esters. Among these conditions may be mentioned (1) excessive peristalsis of the intestine, as in diarrhea and dysentery, (2) intestinal colic and enterospasm, (3) pylorospasm, (4) spastic constipation in which there was a tonic spastic condition of the intestine, (5) biliary colic, (6) ureteral or renal colic, (7) vesical spasm of the urinary bladder, (8) spasmodic pains originating from the contraction of the seminal vesicles, (9) uterine colic, (10) arterial spasm, including hypertension or high blood pressure, (11) bronchial spasm. It has been our aim in this study to shed some light, if possible, on the mechanism by which these conditions are relieved, and to ascertain the concentration of the drug in the blood necessary to produce the desired results.

*From the Pharmacological Laboratory of the University of Cincinnati Medical School, Cincinnati, Ohio.

Respiration
Rt. Carotid
Benzyl Benzoate (Sat. H2O Emulsion)
Dog 13 11. Ether
Base line 6 seconds

Fig. 1.

It will be noted that the first four clinical conditions listed above refer to increased activity or increased tonus of the intestinal tract. We have therefore felt it desirable to study the nature of the relaxation produced by the benzyl benzoate in these conditions. Fig. 1 shows the respiration, right carotid pressure and pylorus contractions in a dog weighing 13 kilos. Near the beginning (left) of the tracing 2 c.c. of benzyl benzoate solution was injected into the femoral vein. This produced no appreciable change in the rate or amplitude of either the pyloric contractions or the respiration. There is perhaps

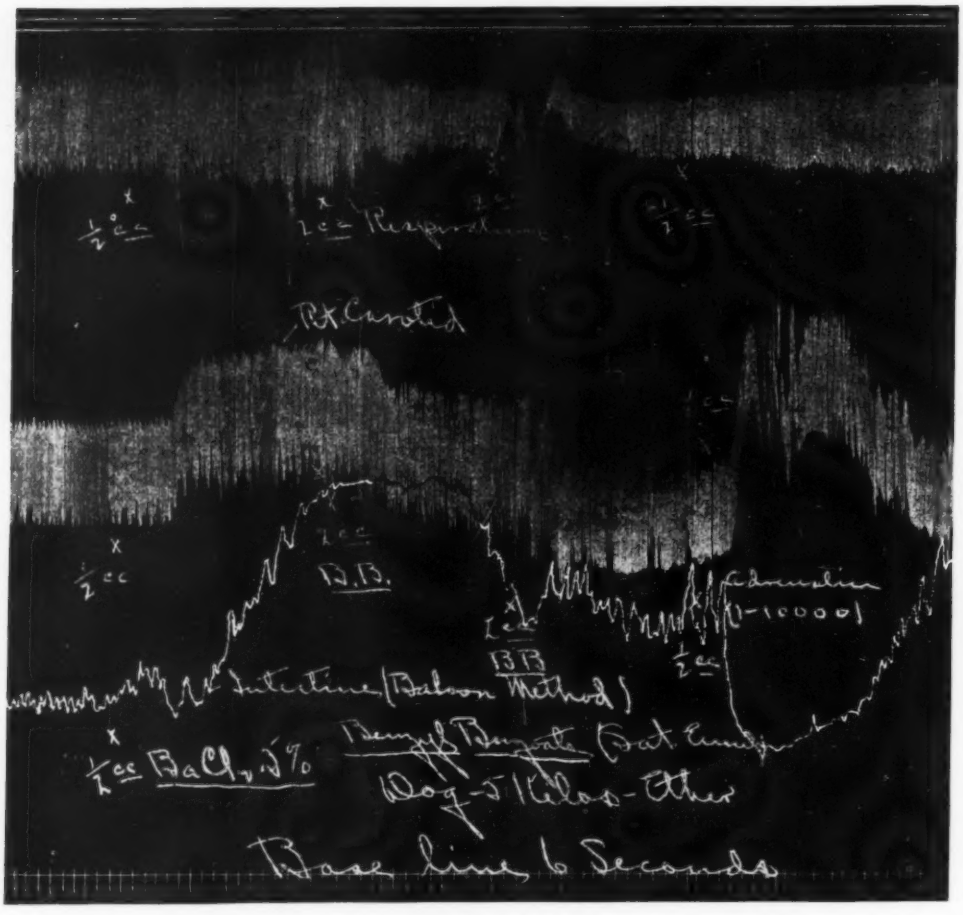


Fig. 2.

a slight change in the blood pressure tracing. Following this 4 c.c. of the benzyl benzoate solution was injected and this produced a depression of the respiration, an obvious fall in blood pressure and perhaps a very slight inhibitory effect on the pyloric contractions. An injection of 1 c.c. of adrenaline (1-10,000) was now given as a check on the technic of the experiment. This produced a very obvious, but rather brief, inhibition of the pyloric contractions. When the animal again returned to normal a further injection of 6 c.c. of benzyl benzoate was given. After a brief interval this dosage produced

complete relaxation of the pylorus, but simultaneously it caused the death of the animal, apparently by central respiratory paralysis. This tracing shows very well the progressive effects of small, medium and large doses of the drug. The marked slowing of the heart following the 4 c.c. injection should be noted.

In order to get further information regarding the action of the drug on the intestinal walls we carried out a series of experiments as illustrated in Fig. 2. In this case a small rubber balloon (finger cot) filled with water was placed within the intestinal lumen and connected by rubber tubing with a burette which carried a stopper in the upper end. From the stopper a rubber

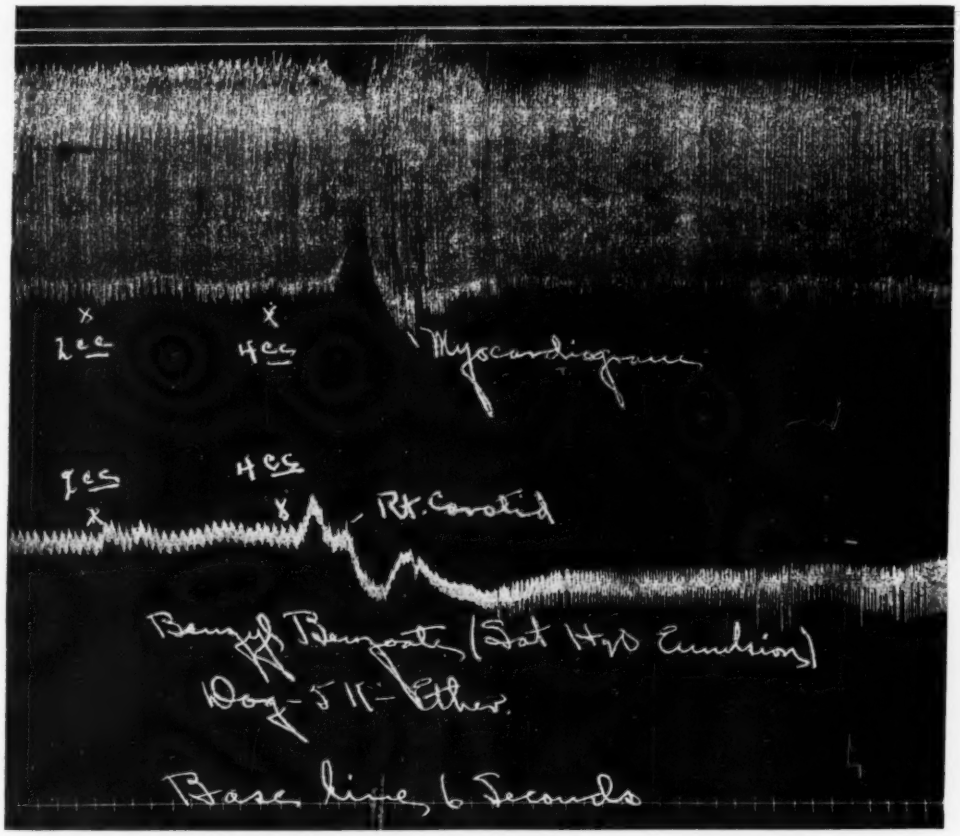


Fig. 3.

tube led to the recording tambour. The water filled the balloon and reached about two inches up in the burette, the upper part of which was filled with air. Thus contractions which lessened the lumen of the intestine caused the writing point of the tambour to rise, and relaxation of the gut showed a fall in the kymograph tracing. In the beginning a small dose (½ c.c. of .5 per cent solution) of barium chloride was injected to stir up marked contractions of the intestine. The marked rise in tone of the intestine is well shown in the tracing. When the circular musculature of the gut was well contracted an injection of 2 c.c. of benzyl benzoate was given. This depressed the respira-

tion and slightly lowered the blood-pressure, but produced no immediate change in the tone of the intestine. After an interval of about one minute, however, there occurred a relaxation of the intestine and at this point a further injection of 2 c.c. of the drug was given. This apparently slightly increased the tonus of the intestine and was followed by a series of small, irregu-

Fig. 4.

lar contractions. As a check on the technic of the experiment an injection of ½ c.c. of adrenalin was finally given. This produced an immediate and complete relaxation of the gut. It will be noted that the second injection of benzyl benzoate greatly depressed the respiration, but both injections of benzyl benzoate taken together were very much less effective in lowering the tone

of the intestinal musculature than was the ½ c.c. of 1-10,000 adrenaline solution.

The injection of a sufficiently large dose of benzyl benzoate is followed by a prompt, pronounced and prolonged fall of blood pressure. From the nature

Fig. 5.

of the blood pressure curve produced, we early conceived the idea that the heart played an important part in this lowering of the arterial tension. Fig. 3 shows a tracing taken directly from the heart (upper curve; myocardiograph)

and the right carotid pressure tracing. It will be noted that 2 c.c. of benzyl benzoate gave little or no change, but that 4 c.c. produced the characteristic fall in blood pressure. It is obvious that this fall in pressure is closely associated with the changes in the amplitude and force of the heart beat. But in order to analyze this point still further we present Fig. 4 in which the drum

Fig. 6.

was made to revolve much more *rapidly* than in Fig. 3. Fig. 4 shows that 3 c.c. of the drug was followed by a fall in blood pressure, but that the heart at the same time became weak and after an interval came near stopping in diastole. After a prolonged series of feeble, irregular beats, however, the heart again recovered slightly, and the blood pressure finally became somewhat more

regular. These changes in the blood pressure are obviously due to a weakening action of the drug on the heart. It should be noted that this animal was receiving artificial respiration.

Fig. 5 shows the action of benzyl benzoate on the respiration, spleen volume and blood pressure of a dog weighing 6.5 kilos. In order to produce a tonus in the spleen an injection of 1 c.c. of .5 per cent barium chloride was given at the beginning of the tracing. The contraction of the spleen became very marked and this was followed after a time by some relaxation. Four c.c. of benzyl benzoate was now injected in order to see whether or not the drug would relax the spleen. It will be noted that the spleen (oncometer) tracing again dips

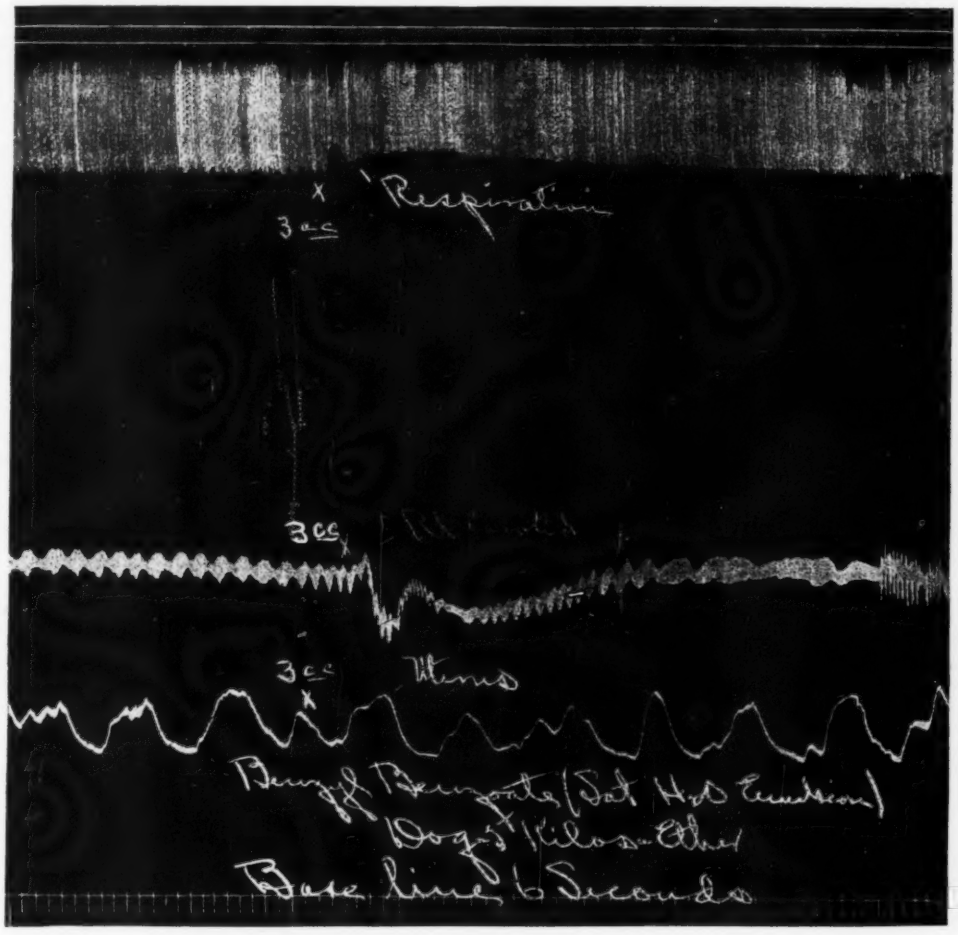


Fig. 7.

even lower than it did following the marked contraction produced by the barium chloride. But the causes of these two spleen contractions are, however, exactly the opposite of each other. In the first, the shrinkage of the spleen volume represents an *active contraction* of smooth muscle within the spleen. The second contraction is passive, and is due to the profound fall in blood

pressure, and perhaps, somewhat to the asphyxia which followed the injection of the benzyl benzoate. Artificial respiration was started at "1" and stopped at "2." Although the blood pressure again returned to a high tension (continued action of the barium) and the spleen tracing also marked a low level (also barium contraction), still there is no evidence that the benzyl benzoate exercised any relaxing action on the spleen, although the dosage was perhaps sufficient to have killed the animal if artificial respiration had not been given. Under artificial respiration animals can withstand very much larger doses of the drug without dying, than is possible under natural respiration. This indicates that the animals do not die of thrombosis as might be suspected from the rather unsatisfactory character of the drug for intravenous injection. The

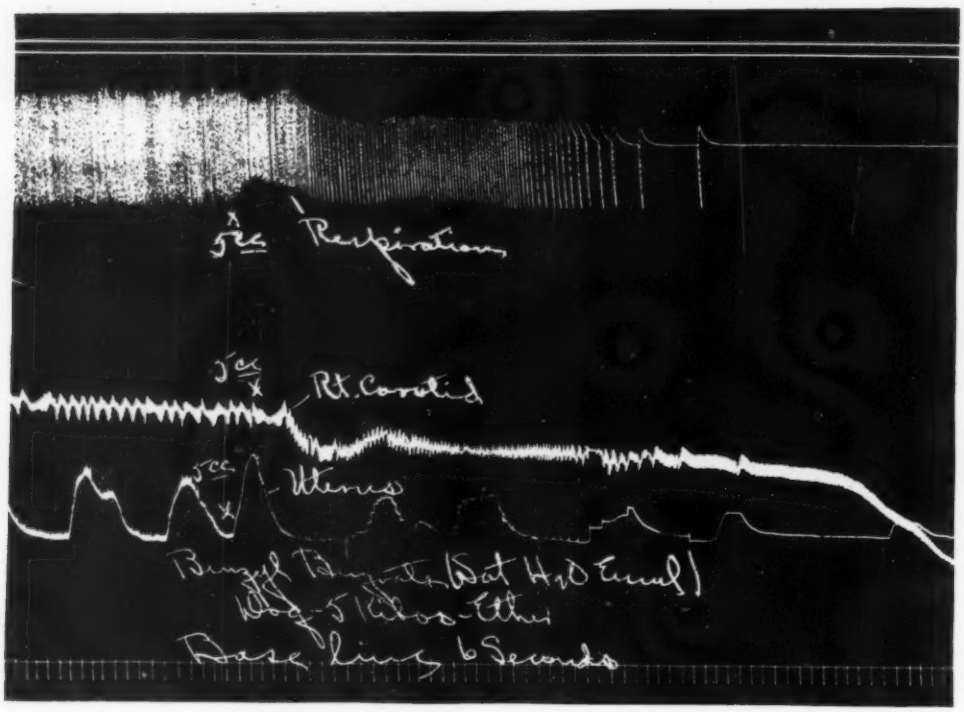


Fig. 8.

final recovery of the heart and circulation, if artificial respiration be maintained, shows that no permanent thrombi are lodged in any vitally important vascular areas.

Fig. 6 shows the respiration, kidney volume (oncometer) and blood pressure. In the beginning 4 c.c. of benzyl benzoate solution was injected. Slight effects were produced in all three tracings. The small shrinkage in kidney volume is obviously secondary, and due to the fall in blood pressure. If the arterioles themselves in the kidney had dilated the volume record would have risen, as occurs after the constriction produced by the 1 c.c. injection of adrenalin (at the center of the tracing) begins to wear off. The shrinkage of the kidney volume after the adraneline is active and is due to the adrenaline stimulating

the myoneural junctions of the vasoconstrictor nerves in the renal arterioles. Following the adrenaline a further dose of 7 c.c. of benzyl benzoate was given. The results of this were exactly analogous to those produced by the 4 c.c., but were correspondingly more pronounced.

The recently suggested use of benzyl benzoate in clinical conditions presumably dependent on excessive or abnormal contraction of the uterus[3] indicated that the drug would probably produce relaxation of this organ. Figs.

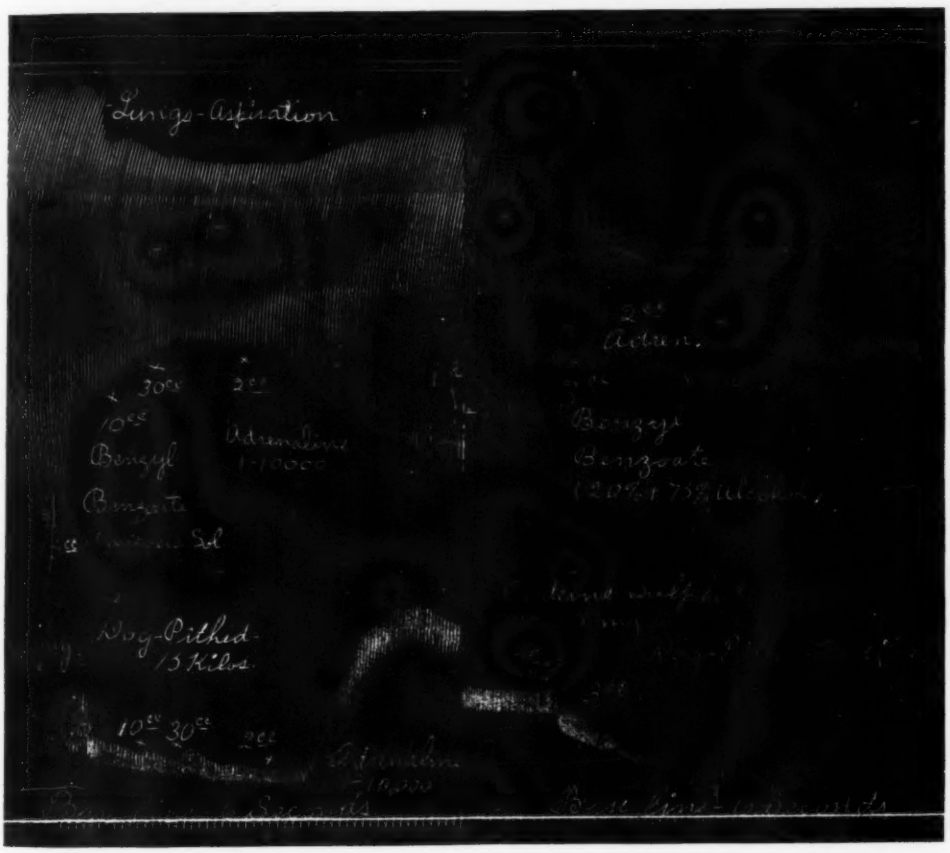


Fig. 9.

7 and 8 show the results we have obtained in the study of the action of the drug on this organ. In Fig. 7 an injection of 3 c.c. was made and produced very obvious results on the respiration and blood pressure. On the uterus, however, the results are very slight. Fig. 8 also shows the effects of an injection of 5 c.c. of the drug. This dosage finally stopped the uterine contractions, but not until the animal had died. Obviously one could not use such large amounts clinically. In these tracings the uterus remained *in situ* and great care was used not to disturb its innervation or blood supply, and to keep the organ warm and moist by closing the abdominal wall and covering the recording apparatus with the intestines (see Jour. Lab. and Clin. Med., 1917, iii, 63). In regard to these two records, however, it should be stated that it is often

difficult to secure entirely satisfactory tracings of the uterus *in situ* in dogs, and we should not be inclined to lay too great emphasis on these observations without checking the results by a considerable number of experiments on animals of different species, which we have not as yet had an opportunity to do. In our present experiments the uterus had previously been roused to increased activity by the injection of pituitrin.

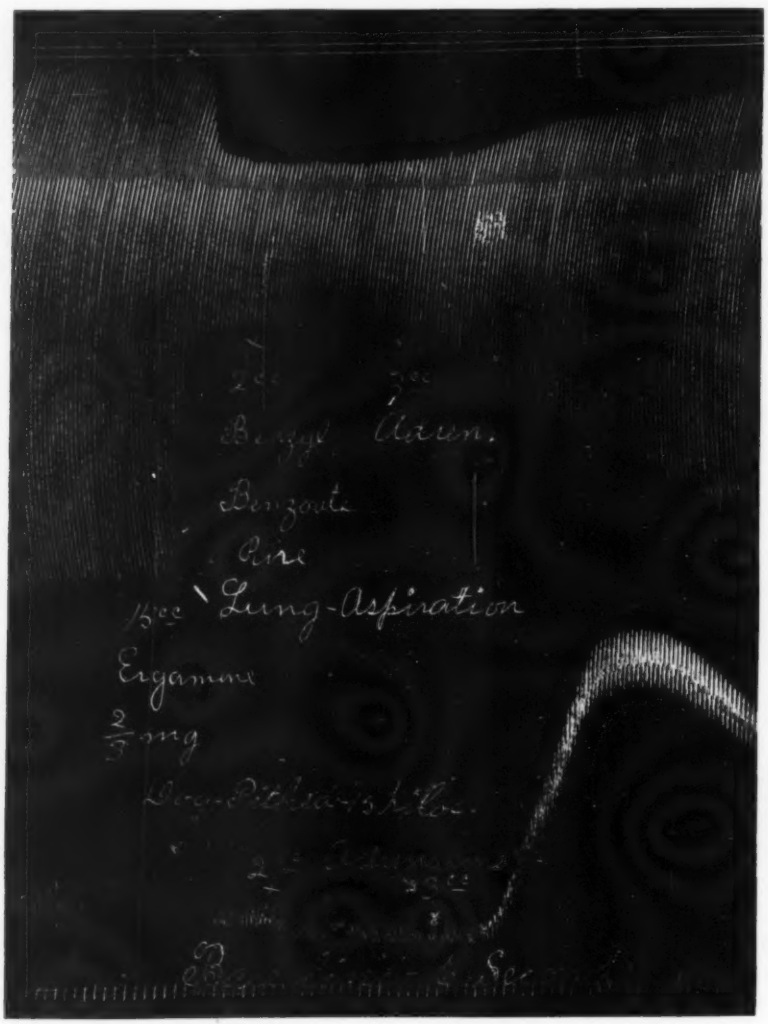


Fig. 10.

One of the most important clinical uses suggested for benzyl benzoate is in the treatment of bronchial asthma.[4] Fig. 9 represents two tracings taken from separate dogs and mounted together. They show the action in spinal dogs of histamine (B-iminazolylethylamine, "ergamine"), benzyl benzoate, codeine and adrenaline on the bronchioles as recorded by a special aspiration method (see Jour. Pharmacol. and Exper. Therap., 1914, vi, 57; also Jackson's

Fig. 11.

Experimental Pharmacology, C. V. Mosby Co., 1917, St. Louis, p. 287). In these tracings (also Figs. 10 and 11) a shortening of the amplitude of the lung record means contraction of the bronchioles, and increase in the amplitude of the lung tracing shows dilatation of the bronchioles. In Fig. 9 the left hand record shows a contraction of the bronchioles produced by the intravenous injection of $\frac{2}{3}$ milligram of ergamine. This led to a bronchial contrac-

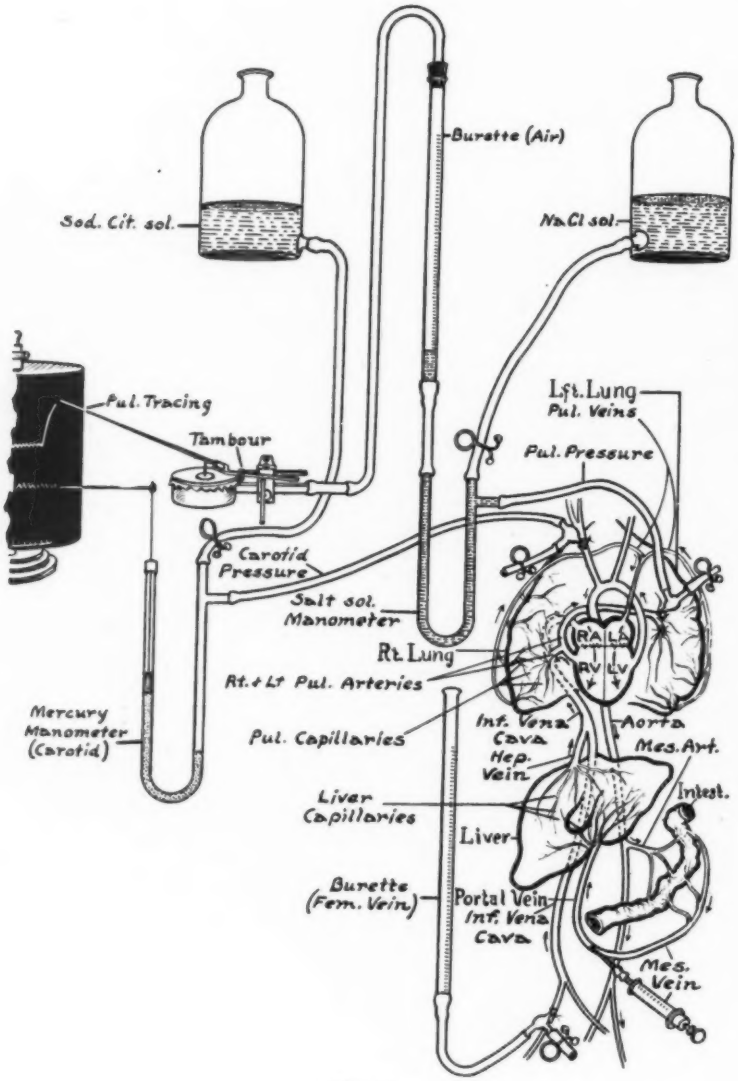


Fig. 12.

tion which 10 c.c. and, later 30 c.c. of an aqueous solution of benzyl benzoate did not relax. An injection of adrenaline (2 c.c.) produced prompt bronchial dilatation. In the right hand tracing codeine was used to produce the initial bronchial contraction and then 3 c.c. of the 20 per cent benzoate made up in 75 per cent alcohol was injected intravenously. The dog weighed 8 kilos and

it would appear that this dosage should certainly have caused dilatation of the bronchioles. This did not occur, however, and as a further check on the technic of the experiment 2 c.c. of adrenalin was injected. A bronchial dilatation followed although the heart stopped beating (from the effects of the codeine and benzyl bezoate). Fig. 10 shows that 2 c.c. of *pure* benzyl benzoate (made by the Harmer Laboratories) did not produce the slightest indication of a bronchial dilatation following a contraction produced by "ergamine." Adrenaline, however, caused a marked dilatation. This seems to indicate definitely that benzyl benzoate does not cause a bronchodilatation in intact (pithed) dogs under the conditions obtaining in such experiments as we have here carried out. Fig. 11 shows three separate tracings mounted together. From the legends it will be seen that benzyl benzoate did not produce satisfactory bronchodilatation in either instance.

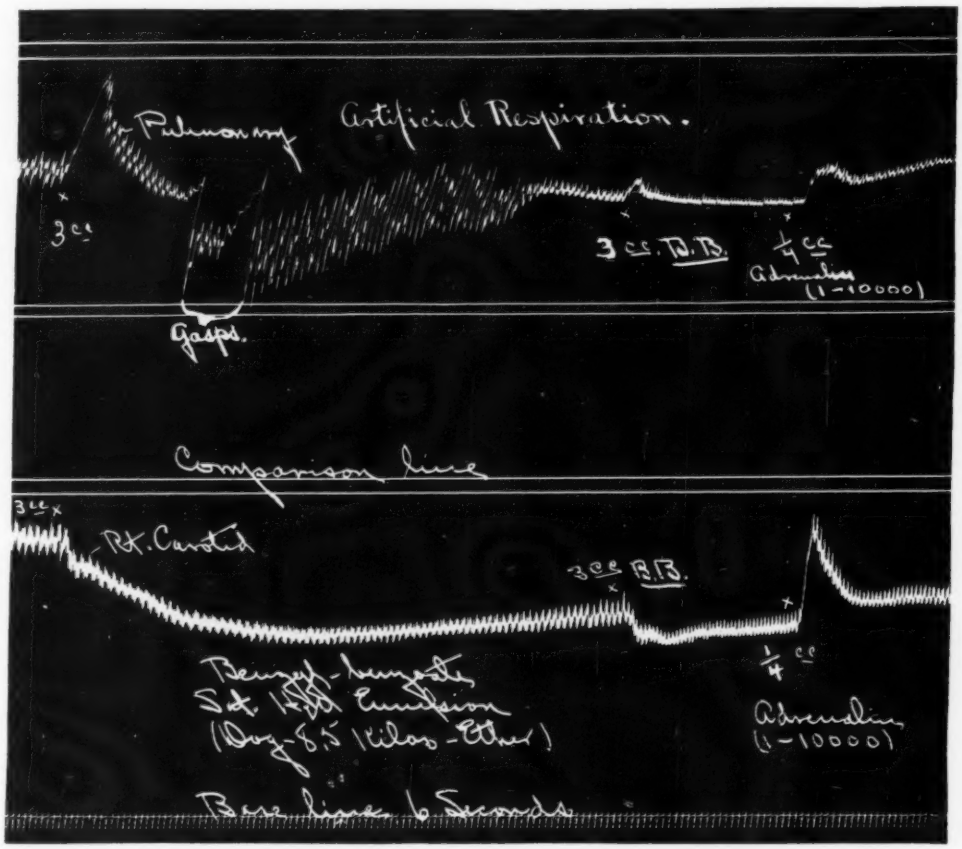


Fig. 13.

The possibility that benzyl benzoate might be used clinically in pulmonary hemorrhage in cases of tuberculosis led us to investigate the action of the drug on the pulmonary blood pressure. The method we have used for recording the pulmonary arterial pressure is indicated in Fig. 12. (See Jour. Lab. and Clin. Med., 1920, vi, 1). In the diagram it will be seen that a cannula tied into

the left pulmonary artery connects with a water manometer, the distal end of which is joined to the lower end of a burette. The manometer and tubes to the artery (and the cannula) are filled with normal salt solution. The upper end of the burette is connected with a recording tambour which writes on the

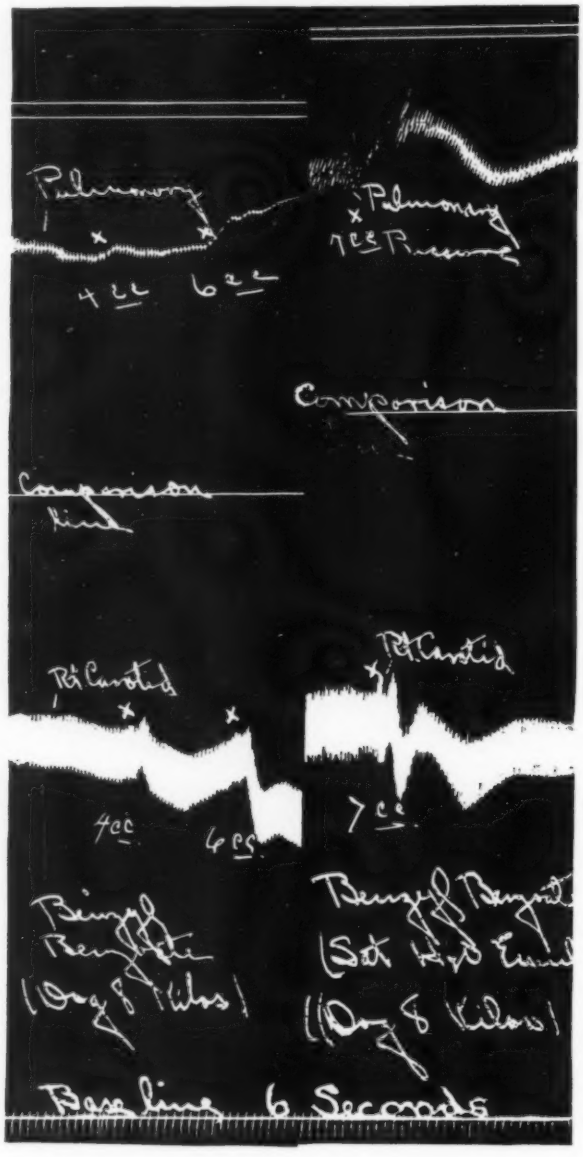


Fig. 14.

drum. The tambour, the upper part of the burette and the connecting tubes are filled with air. By this method the pulmonary blood pressure record represents a magnification about 150 times greater than would be recorded by a mercury manometer. The variations in the pulmonary tracings should, there-

fore, be reduced about 150 times in amplitude in order to compare them directly with the associated carotid tracings which, in our records, were made with a mercury manometer. Fig. 13 shows the results we have observed on the pulmonary arterial pressure. Near the beginning of the tracing 3 c.c. of benzyl benzoate was injected and a typical, prolonged fall in the carotid pressure, was obtained. In the pulmonary pressure, however, there was produced at first a sharp temporary rise which was succeeded by a few gasping movements. But on the average there was extremely little variation in the pulmonary pressure, either in the way of a rise or a fall. Later a 3 c.c. injection of benzyl benzoate was given again. The fall produced in the carotid pressure was again quite typical, but the pulmonary pressure showed only a very slight, transient rise. A small injection of adrenaline was finally given in order to check up the accuracy of the technic in the experiment. Fig. 14 shows two short pulmonary tracings (mounted together). Here again the drug produced only the slightest changes in the pulmonary pressure. In studying these tracings one must, of course, constantly bear in mind the greatly increased magnification of the pulmonary over the carotid records. It is obvious that if the drug should act in clinical cases in a manner at all analogous to that in which it has behaved under these experimental conditions, it could be of no use in the matter of treatment of hemoptysis in pulmonary tuberculosis.

REFERENCES

[1]Macht, David, I.: Jour. Pharmacol. and Exper. Therap., 1918, xi, No. 6, p. 421.
[2]Macht, David I.: Jour. Am. Med. Assn., Aug. 23, 1919, pp. 599-601.
[3]Litzenberg, Jennings C.: Jour. Am. Med. Assn., Aug. 23, 1919, pp. 601-603.
[4]Macht, David I.: Southern Med. Jour., July, 1919, xii, No. 7, p. 367.

A REPORT OF AN EPIDEMIC OF INFLUENZA IN AN ARMY POST OF THE AMERICAN EXPEDITIONARY FORCES IN FRANCE

By Alan M. Chesney, M.D., and Frank W. Snow, M.D.

(Formerly Officers in the Medical Corps of the United States Army)

A. INTRODUCTION

THE outbreak of influenza which forms the subject of this paper was described in an official report by one of us (Chesney) to the Chief Surgeon, American Expeditionary Forces, France. At the instance of the Surgeon General that report has been revised for publication and forms the basis of this paper. It was thought that a report of this particular epidemic would be of interest in view of the fact that it seemed to throw some light on one or two problems related to the epidemiology of influenza, more especially the question as to whether or not the virus of influenza acquires increased virulence by successive passage through human hosts.

B. SITE OF THE EPIDEMIC

The outbreak which it is proposed to describe occurred in the Post of A. P. O. 704, American Expeditionary Forces, France. This post was a permanent artillery camp of the French Army, located at LeValdahon in the department of the Doubs. It comprises a tract of rolling land large enough to provide sufficient room for artillery range, and in addition possesses a number of permanent two-story barracks buildings of cement construction. The use of this camp was loaned to the American Army and after it had been taken over by the latter a number of wooden barrack buildings were erected for purposes of instruction and for additional barrack and stabling space.

During the summer and autumn of 1918 American artillery brigades were ordered to this post for periods of from four to six weeks for instruction. It frequently happened that while one brigade occupied the post and was receiving instruction another brigade was billeted in neighboring towns and was awaiting the opportunity to enter the post for the same purpose. It was the custom of one brigade to enter the post as soon as its predecessor had left. Thus every four to six weeks there occurred a marked change in the population of the post.

In addition to the brigades which occupied the post there was a permanent post personnel constantly present which consisted of troops belonging to the Cavalry arm, the Engineer Corps, Quartermaster Corps, and Medical Corps, which were engaged in the administration of the post. The permanent personnel amounted to about 500 and was fairly constant. The total population of the post when a brigade was billeted in it varied from 3500 to 5000. The post hospital, Camp Hospital No. 12 received patients from all the organ-

izations in the post as well as from those in the surrounding billeting area. One of us (Snow) was Commanding Officer of the hospital during the period of the epidemic and Post Surgeon from about August 1st, the other (Chesney) was detailed to make a special study of the epidemic.

The frequent changes in the population of the post brought about by the short stay of each brigade exercised considerable influence upon the course of the epidemic. Indeed the history of the epidemic resolves itself into distinct periods corresponding to the various brigades which entered the post and it will be profitable to approach the events from that point of view.

C. HISTORY OF THE EPIDEMIC

1. First Period, June to July 27th, 1918

The first period covers the duration of the stay of the 5th Field Artillery Brigade in the post. During this interval a respiratory infection diagnosed as influenza made its appearance in troops billeted there. It was largely confined to troops belonging to the 5th Brigade. Thus of 77 cases diagnosed as influenza, bronchopneumonia or lobar pneumonia admitted to the post hospital between July 1st and July 27th from organizations actually barracked in the post, 68 developed in organizations of the 5th Brigade. In Table I is shown the distribution of these cases among the commands in the post.

TABLE I

DISTRIBUTION OF CASES OF RESPIRATORY DISEASE IN ORGANIZATIONS BARRACKED IN POST OF A. P. O. 704, JULY 1-27, 1918

ORGANIZATION	NUMBER OF CASES ADMITTED TO HOSPITAL
5th Artillery Brigade	
Brig. Hdqrts. Co.	17
5th Trench Mortar Battery	12
19th Field Artillery Reg't	
Battery A.	2
Battery B.	2
Battery C.	2
Battery D.	1
Officers	1
20th Field Artillery Regt.	
Headquarters Co.	3
Supply Co.	1
Sanitary Detachment	1
Battery A.	1
Battery B.	4
Battery C.	5
Battery D.	2
Battery E.	2
Battery F.	2
Officers	3
21st Field Artillery Regiment	
Headquarters Co.	1
Battery B.	1
Battery C.	2
Battery E.	2
Officers	1
Permanent Post Organizations	
Troop C. 2nd Cavalry	5
Medical Detachment Camp Hospital No. 12	2
Unattached Officers	2

In compiling these statistics no differentiation has been made between cases of influenza, acute bronchitis, bronchopneumonia or lobar pneumonia. They are all grouped under the heading "Acute Respiratory Disease." As a matter of fact most of the cases were cases of influenza.

From Table I it is seen that certain organizations were affected more than others, notably Headquarters Company of the brigade and the 5th Trench Mortar Battery. The disease was relatively mild at that time. Just how it made its way into the post is uncertain, but it was known to be prevalent among the civilian population of Besancon, a city located some 30 kilometers away, and the troops were permitted to visit that city on pass. During the month of July, while the 5th Brigade occupied the post the 58th Field Artillery Brigade occupied billets in surrounding towns and was waiting to enter the post for its period of training. The withdrawal of the 5th Brigade marks the end of the first period of the epidemic and the beginning of the second.

2. SECOND PERIOD, JULY 27TH TO AUG. 23RD

The second period, lasting from July 27th to August 23rd, represents the duration of stay of the 58th Field Artillery Brigade in the post. This Brigade was composed of the 122nd, 123rd, and 124th Field Artillery Regiments, the 108th Ammunition Train, the 108th Trench Mortar Battery and the 108th Motor Ordnance Repair Shop. During the time that these organizations occupied billets in the vicinity of the post, a period of four weeks before their entry, no cases of influenza, bronchitis or pneumonia occurred in them.

The 123rd and 124th F. A. Regiments began to enter the post on July 27th, and about three days were consumed in the process of moving in. The 122nd Regiment did not enter the post at all, but remained in billets in a town about one kilometer distant and sent men to the ranges of the post every day for instruction in firing. The troops of the 123rd and 124th Regiments occupied barracks at the southwestern end of the post and slept upon mattresses and pillows that had been used by outgoing troops of the preceding brigade. No general attempt was made to clean these barracks before the entry of the new brigade.

Several days after the 58th Brigade had entered the post, cases of influenza began to appear in units of that brigade and the number of such cases increased rapidly. The distribution, among the various commands in the post, of cases of acute respiratory disease that occurred betwen July 27th and August 23rd inclusive, and were admitted to the post hospital, is shown in Table II.

Table II shows that of all cases of acute respiratory disease admitted to the post hospital during the period of July 27th to Aug. 23rd, the great majority came from the 123rd and 124th Field Artillery regiments. In Figs. 1, 2, and 3 are exhibited curves which show the daily number of cases of acute respiratory disease admitted to hospital from these organizations, together with those from the 122nd regiment, during the entire time of their stay in the post as well as for the previous four weeks during which they were in towns in the neighborhood. These curves show that there were no cases of influenza

or other respiratory infection in these regiments during the first four weeks of July, while all three were billeted in nearby towns and that the 123rd and 124th F. A. regiments, which occupied the camp during the last few days of July and the first three weeks of August, had a number of cases of influenza following their entrance into the post, whereas the 122nd F. A., which remained

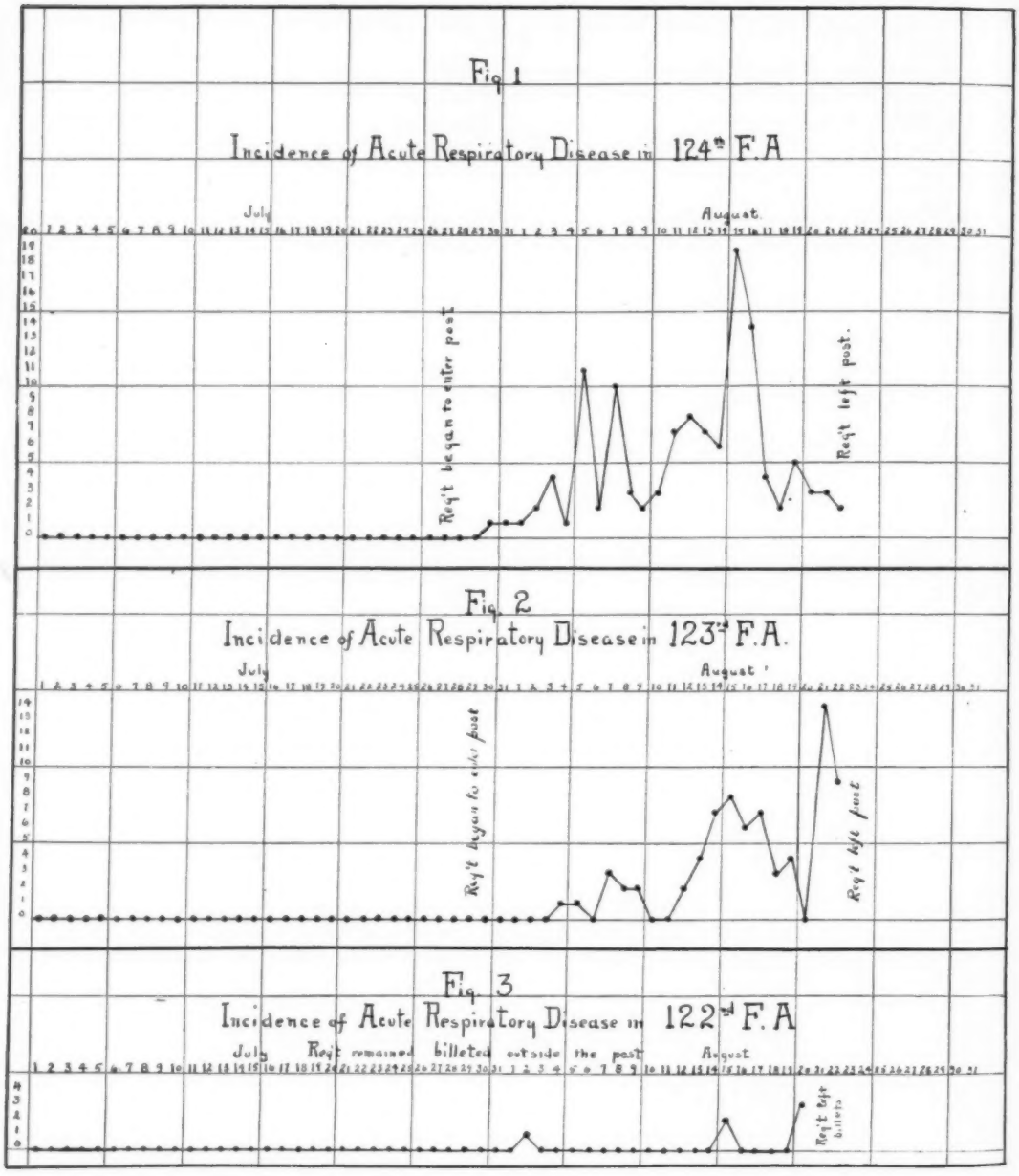
TABLE II

DISTRIBUTION OF CASES OF ACUTE RESPIRATORY DISEASE OCCURRING IN COMMANDS BARRACKED IN POST A. P. O. 704, A. E. F. DURING JULY 27th TO AUG. 23rd, 1918

ORGANIZATION	NUMBER OF CASES ADMITTED TO HOSPITAL
58th Field Artillery	
Brigade Headquarters Company	0
108th Ammunition Train	1
108th Trench Mortar Battery	5
108th Motor Ord. Repair Shop	2
123rd Field Artillery Reg't	
Headquarters Co.	5
Supply Co.	6
Ordnance Det.	2
Sanitary Det.	5
Battery A.	11
Battery B.	3
Battery C.	3
Battery D.	19
Battery E.	6
Battery F.	6
Officers	7
124th Field Artillery	
Headquarters Co.	21
Supply Co.	0
Ordnance Det.	0
Sanitary Det.	10
Battery A.	14
Battery B.	2
Battery C.	12
Battery D.	2
Battery E.	33
Battery F.	30
Officers	4
Sixth Field Artillery Brigade	
*Brigade Headquarters	2
*6th Trench Mortar Battery	1
*Headquarters F. A.	1
*Headquarters Company 11th F. A.	1
*Supply Company 11th F. A.	1
*Ordnance Detachment 11th F. A.	1
*Battery E. 11th F. A.	4
*Battery F. 11th F. A.	2
Post Organizations	
2nd Cavalry	2
309 Q. M. C.	10
Post Engineers	4
Post Ordnance	3
Post Signallers	2
Medical Detachment Camp Hospital No. 12	10
3rd Veterinary Hospital	1
800th Aero Squadron	4
14th Balloon Squadron	1
Officers Unattached	2
	252

*These organizations were sent into the post in advance of the Sixth Brigade itself for preliminary instruction.

billeted in LeValdahon, a kilometer away, had only six cases of influenza during the entire time, three of which could be fairly definitely traced to the post. This last regiment, which remained outside the camp but sent men to the ranges daily for instruction, served therefore as a satisfactory control



for the regiments which entered the camp. There can be little doubt that the organizations of the 58th Brigade which underwent an epidemic of influenza while in the Post of A. P. O. 704, A. E. F., became infected as a direct result of moving into an infected place. They did not bring the infection with them.

The total number of cases of respiratory infection admitted to the hospital from those organizations of the 58th Brigade located in the post

amounted to 200. The total strength of these same organizations on August 1st was approximately 3000, so that during their stay in the post about 6.5 per cent of the troops belonging to them were rendered unfit for duty on account of acute respiratory disease.

A spot map (see Fig. 4) designed to show the location of cases in camp, demonstrated that the disease was confined largely to the southwestern group of barracks and that there were relatively few cases in the northeastern and southeastern groups. The southwestern group of barracks which was occupied by the 123rd and 124th Regiments, as well as by some of the permanent personnel of the post, had previously been occupied by those organizations of the preceding (Fifth) brigade in which influenza had been most prevalent. The northeastern group was occupied by some permanent post organizations among which a few cases only occurred and the southeastern group was occupied by some organizations of the 58th Brigade as well as by some detachments of the 6th Brigade which had been sent into the post in advance of the brigade itself for preliminary instruction.

Once the epidemic had commenced in the 58th Brigade it assumed the characteristics of a battery infection, being fairly well restricted to certain batteries, while other batteries were almost entirely free from the infection. (See Table II.) It appears probable that the disease was spread among the men by direct contact in the barracks, where there was ample opportunity for such contact to occur. Inspection of the barracks showed that the beds were frequently less than one foot apart and that no consistent attempt at head and foot arrangement was made. The estimated floor space per man varied from 31 to 50 square feet, and was usually less than 40 square feet.

On August 22nd the 124th F. A. vacated its barracks and on the following day the 123rd left the post. Both of these regiments swept out their barracks before leaving. Their departure marks the end of the second period of the epidemic.

3. THIRD PERIOD, AUGUST 23RD TO NOVEMBER 8TH, 1918

The third period may be said to extend from August 23rd, the date of departure of the 58th Brigade, to November 8th, the date of departure of the last of the brigades sent to the post for instruction during the Summer and Autumn of 1918. This period represents the duration of stay of the 6th and 156th Artillery Brigade in the post.

During the first three weeks of August, while the 58th Brigade was occupying barracks in the post, the 6th Field Artillery Brigade was billeted in nearby towns, awaiting its turn to enter the post for instruction. Several units from the brigade were actually sent into the post in advance of the brigade itself and occupied barracks in the southeastern portion of the post during the time that the 58th Brigade was having its epidemic. Table II shows that thirteen cases of acute respiratory infection occurred among these advance units of the 6th Brigade after they entered the post. The remaining units of the brigade during their stay in billets outside the camp were almost entirely free from acute respiratory disease, not more than twelve cases being

admitted to the hospital from these organizations during the period preceding their entry into the post.

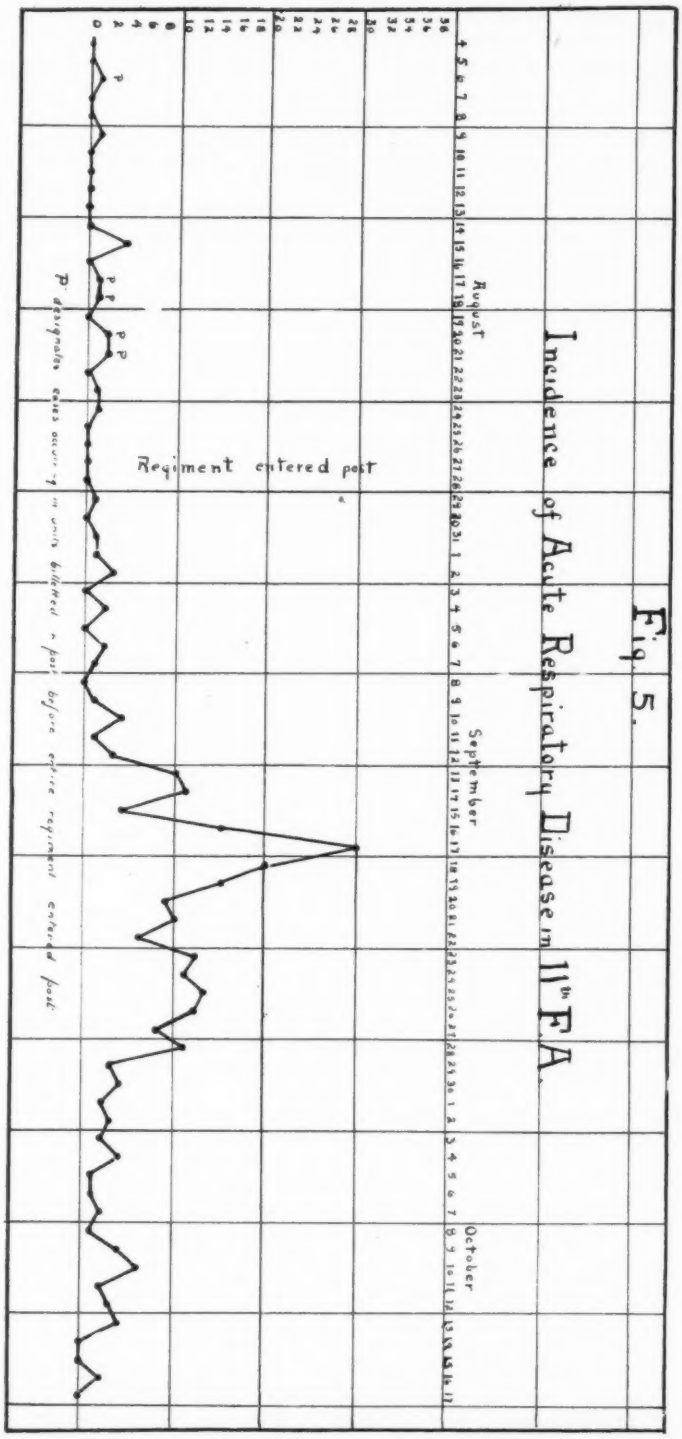


Fig. 5.
Incidence of Acute Respiratory Disease in 11th F.A.
August
September
October
Regiment entered post
P designates cases occurring in units billeted in post before entire regiment entered post

When it became known that the 58th Brigade was about to leave the post and that the 6th was to enter, efforts were made by the Post Surgeon at that time (Snow), assisted by the medical officers of the 6th Brigade, to prevent the occurrence of an epidemic of influenza in the latter brigade. The situation was explained to the Commanding General of the 6th Brigade and it was recommended to him that his brigade be kept in billets in the surrounding towns rather than be permitted to enter the post, or, if the brigade did enter the post, that the men be put under tents rather than in the barracks of the post. In view of the experience with the 58th Brigade it was predicted that if the 6th Brigade entered the barracks vacated by the former brigade the latter would, in all probability, experience an epidemic of influenza as severe as, or even more severe than, that undergone by the 58th Brigade. These recommendations were not accepted. However, it was decided that a week should be permitted to elapse between the departure of the 58th and the entry of the 6th Brigade into the post, and that during that interval the barracks should be thoroughly scrubbed with soap and water and whitewashed, and the pillows and mattresses aired in the sunshine. Orders were issued to the incoming troops to use sacks filled with hay or straw for bedding and not use bedding that had been used by the outgoing brigade. The men were supposed also to be billeted in such a manner that each had at least 40 square feet of floor space.

Immediately following the departure of the 58th Brigade detachments from the 6th Brigade began to carry out the prescribed cleansing measures in the southwestern group of barracks. All beds, bedding and movable fixtures were taken out doors and left in the sunshine for several days.

The walls were washed down with soapy water applied with brooms, the floors were then scrubbed with soap and water and some of the rooms were whitewashed. The work in the southwestern group of barracks was performed by detachments from the 3rd and 78th Field Artillery regiments, which had been selected to occupy those particular barracks. The southeastern barracks were not cleaned by the regiment (11th F. A.) which was to occupy them until several days after that regiment had moved into them. Two batteries of the regiment together with some other detachments from the 6th Brigade had moved into these barracks several weeks previously and thirteen cases of influenza had developed in these units after their entry (See Table II).

On August 27th the 6th Brigade entered the post. During the fortnight following the entrance of the brigade into the post some cases of influenza made their appearance in the three regiments constituting the brigade, but the cases were scattered and the incidence was not such as to constitute a real epidemic until the 10th of September when a large number of men from the 3rd F. A. regiment suddenly came down with the disease. From that time on cases developed with great rapidity in the regiment and two or three days later the disease was epidemic in the other two regiments of the brigade. The permanent post organizations continued to contribute cases of influenza during this time. In Table III is shown the distribution, among the various

commands in the post, of cases of acute respiratory disease admitted to the post hospital during the stay of the 6th Brigade in the post.

Table III shows that of the cases of acute respiratory disease admitted to the post hospital from organizations barracked in the post during the period August 24th to October 21st, 1463 developed in organizations belong-

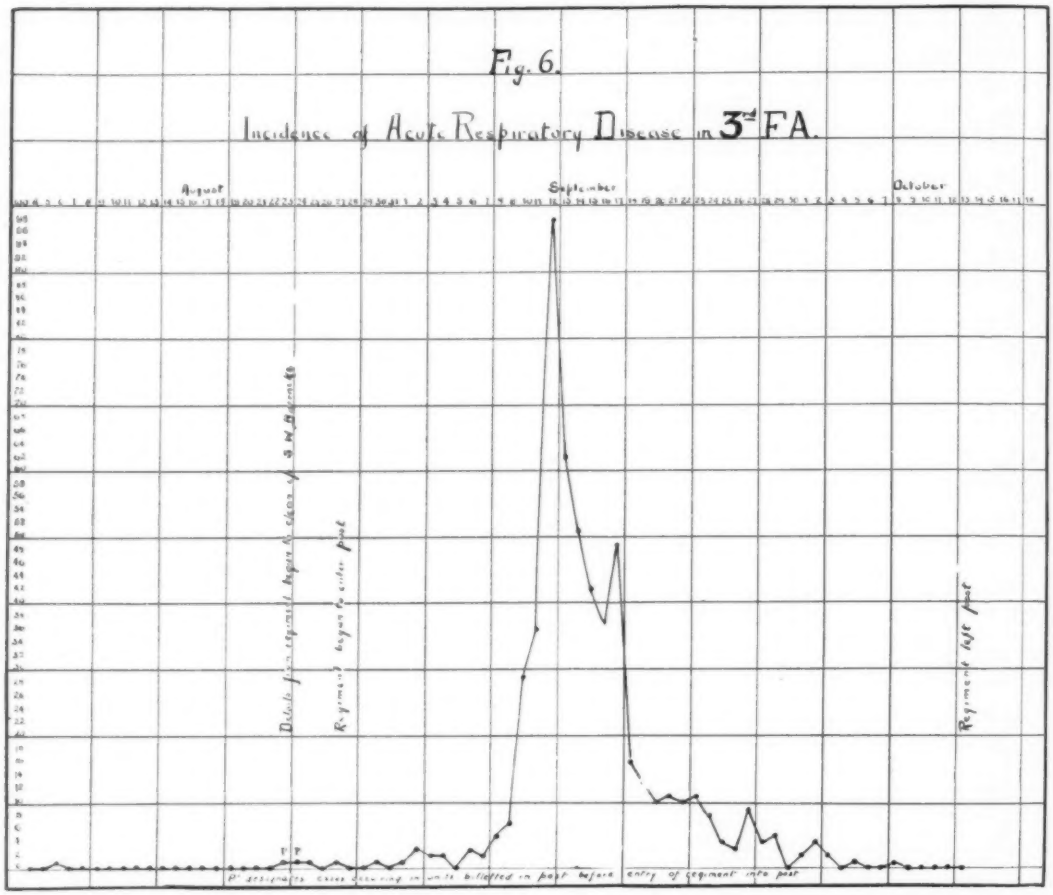
TABLE III

DISTRIBUTION OF CASES OF ACUTE RESPIRATORY DISEASE OCCURRING IN COMMANDS BARRACKED IN THE POST OF A. P. O. 704, A. E. F. DURING THE PERIOD OF AUGUST 24th TO OCTOBER 21st, 1918

ORGANIZATION	NUMBER OF CASES ADMITTED TO HOSPITAL
6th Artillery Brigade	
Brigade Headquarters Co.	9
Brigade Casual Detachment	138
3rd Field Artillery	
Headquarters Co.	71
Supply Co.	60
Ordnance Det.	6
Sanitary Det.	13
Battery A.	74
Battery B.	52
Battery C.	140
Battery D.	63
Battery E.	30
Battery F.	15
Officers	16
11th Field Artillery	
Headquarters Co.	46
Supply Co.	7
Ordnance Det.	4
Sanitary Det.	12
Battery A.	31
Battery B.	28
Battery C.	30
Battery D.	15
Battery E.	55
Battery F.	27
Officers	10
78th Field Artillery Regiment	
Headquarters	49
Supply Co.	50
Ordnance Det.	4
Sanitary Det.	7
Battery A.	103
Battery B.	78
Battery C.	22
Battery D.	34
Battery E.	25
Battery F.	120
Officers	19
Post Organization	
2nd Cavalry	4
309 Q. M. C.	14
Post Ordnance	4
Post Headquarters	10
Camp Hospital	51
Officers Unattached	8
3rd Vet. Hospital	45
375th M. T. C.	1
800th Aero Squad	25
14th Balloon Sq.	3
304th Remount	3
323rd Labor Batt'n	13

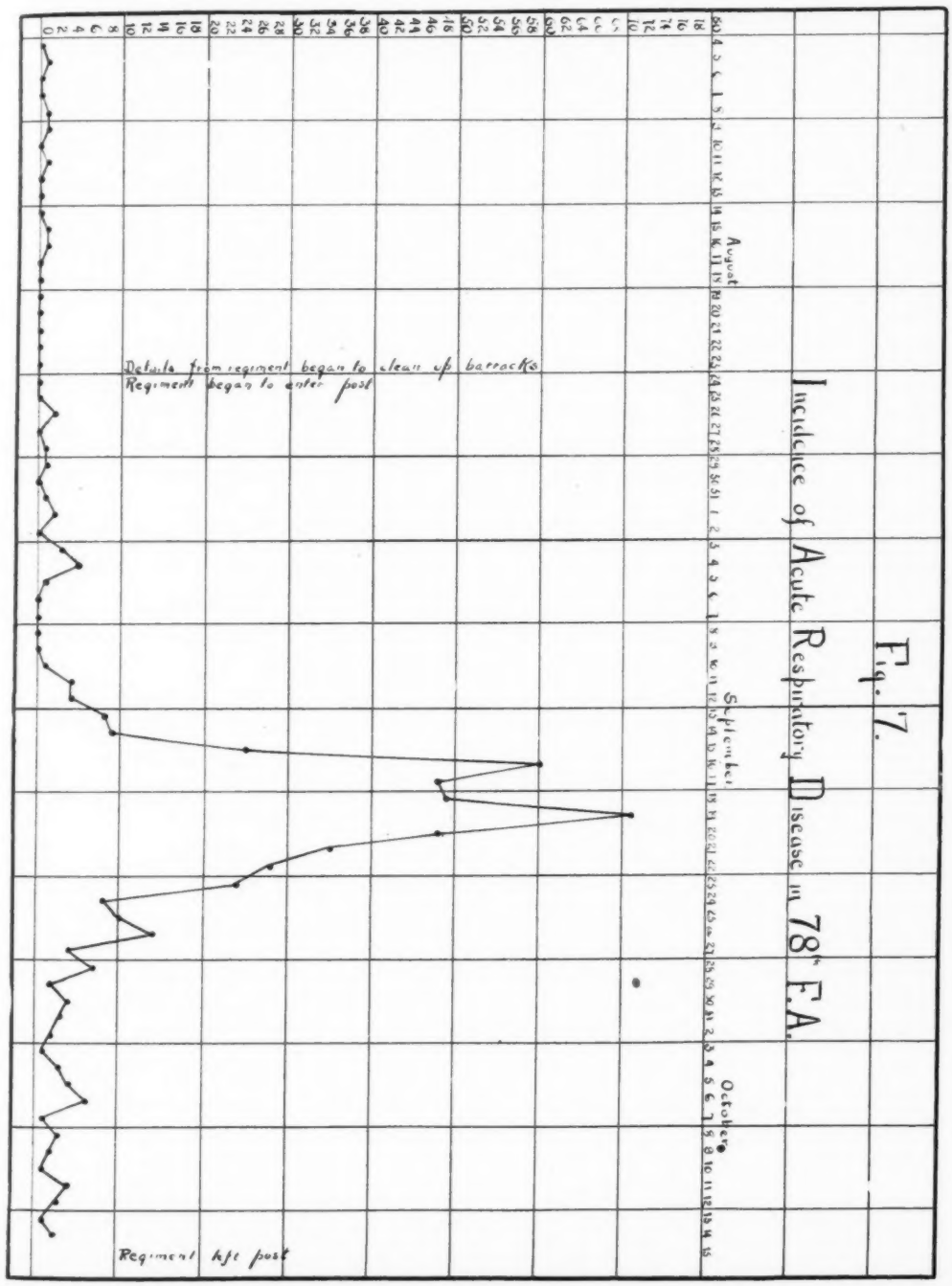
ing to the 6th Brigade and the remaining 181 in permanent post organizations.

In Figs. 5, 6 and 7 are illustrated curves which show the number of cases of respiratory disease admitted to the hospital daily from the three regiments of the 6th Brigade, before their entry into the post. These curves show that these regiments were almost free from cases of respiratory disease previous to moving into camp, and that influenza was not a serious menace until two weeks after their entry into the post.



In Fig. 8 is shown the daily incidence of respiratory disease in organizations barracked in the post during the period July 1st to October 21st. It shows that as successive brigades entered the post there was a progressive increase in the number of soldiers who developed influenza. The epidemic as it manifested itself in the first of the brigades was relatively mild, in the second brigade to enter the post it was more severe and in the third it was more severe than in the other two. Thus in the first brigade to enter the post the morbidity may be estimated at about 1.5 per cent, in the second at about 6.5 per cent, and in the third it reached the high figure of about 34 per cent.

It is seen from Table III that there was considerable variation in the extent to which different organizations were affected. The highest incidence in any one battery occurred in Battery C of the 3rd F. A. regiment and amounted to 141 cases, a morbidity of about 70 per cent. The least incidence in any one battery of the same brigade during the same period was that occurring



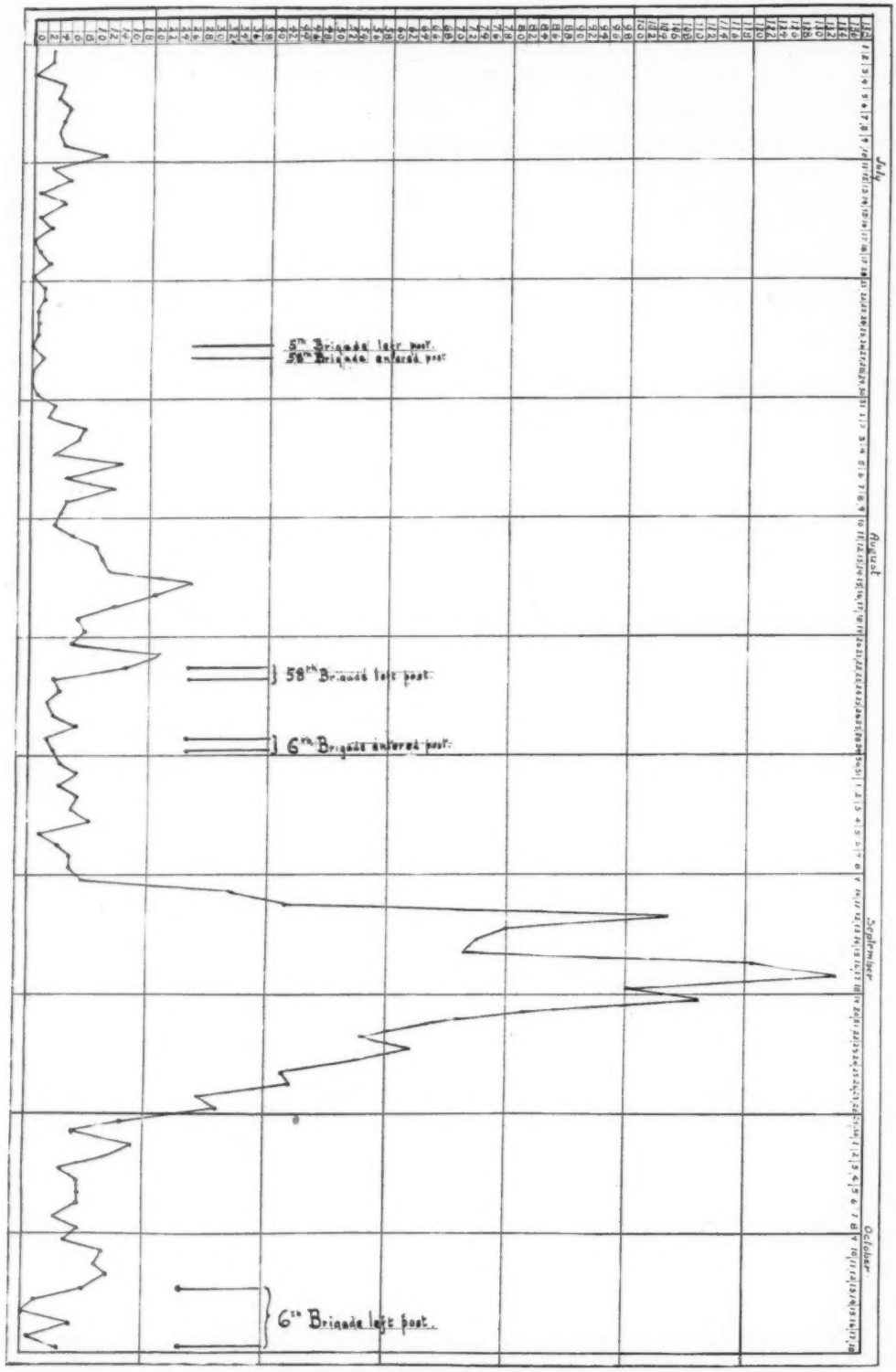


Fig. 8.—Occurrence of Influenza in Organizations Barracked in the Post of A. P. O. No. 704.

in Battery F of the 3rd F. A., which had only 15 cases. There was a tendency for the disease to appear in an organization with what may be termed "explosive violence," a large number of men in a single battery coming down with the disease on the same day, when the battery had previously been almost free from the disease. The greatest number of admissions to hospital from any one battery in one day was 40.

It was impossible to prepare a spot map to show the barrack location of each case occurring during the third period of the epidemic for the reason that the units were frequently shifted about in barracks and this factor made it impossible to trace the individual cases. However, it is worthy of note that the two regiments of the 6th Brigade which suffered the most moved into barracks that had been occupied by the two regiments of the previous brigade in which influenza had been most prevalent. (See Fig. 4.)

Once the disease had become well established, it spread rapidly through the various commands and the epidemic ended apparently by burning itself out. The 6th Brigade began to vacate the post on Oct. 13th and the last regiment left on Oct. 21st. Although the date of the departure of the 6th Brigade marks the end of the epidemic of influenza in the post, it does not mark the end of the epidemic in the vicinity, for shortly after the epidemic of influenza in the 6th Brigade reached its height, influenza began to be epidemic among the troops of the 156th Artillery Brigade which at that time was billeted in surrounding towns.

This brigade arrived in the vicinity of the Post of A. P. O. 704, during the last week of August 1918, having come almost directly from the United States. It had had no influenza previous to its arrival in the nearby towns where it was to be billeted, but about three weeks after arrival in the billeting area influenza began to be epidemic in one of the regiments and within a week the other two regiments had become affected. These regiments were billeted in three separate towns, situated many miles apart, and yet there was not the difference of a week in the date of onset of the epidemic in the three regiments. The epidemic in the 156th Brigade had practically reached its end by the time the 6th Brigade was ready to leave the post, so that no objections to the entry of the former brigade into the post were raised by the medical authorities. The 156th Brigade began to enter the post Oct. 21st, and left on Nov. 8th. Only 28 cases of influenza developed in this organization during its stay in the post. The entire number of cases developing in this brigade during its stay in the neighborhood of LeValdahon amounted to 1048, a somewhat smaller number than that occurring in the 6th Brigade. The general characteristics of the outbreak in this brigade were the same as those in the 6th Brigade.

The total number of cases diagnosed as influenza, acute bronchitis, bronchopneumonia and lobar pneumonia, occurring in organizations barracked within the post of A. P. O. 704, A. E. F. or billeted in the vicinity, during the period July 1st to Nov. 8, 1918, amounted to 3162. By far the majority of these cases developed within the post and were admitted to the post hospital, Camp Hospital No. 12, although a few were cared for in regimental infirm-

aries as long as no complications developed. None of the permanent post organizations escaped infection, those most seriously affected being the camp hospital detachment, the 3rd Veterinary Hospital and the Quartermaster Corps detachment.

A number of cases of influenza developed pulmonary complications. It is difficult to be sure of the exact number of such cases, particularly during the earlier part of the epidemic. From Sept. 1st to Nov. 8th, when respiratory diseases were most prevalent, there were admitted to the Camp Hospital No. 12 or there developed within the hospital, 324 cases of bronchopneumonia or lobar pneumonia. This figure represents about 12 per cent of the total number of cases of respiratory infection that occurred in the vicinity of the post of A. P. O. during the same period. Of these 324 cases of pneumonia (broncho- and lobar) 151 died, giving a case mortality rate of 46 per cent for the lung complications. In addition to the cases with pulmonary complications there were a number of cases in which meningococcus meningitis developed during the period of convalescence from influenza.

D. DISCUSSION

The epidemic of influenza at LeValdahon offered an unusual opportunity for the collection of data which might throw light on the epidemiology of this disease. The exposure, in turn, of large bodies of men of approximately the same age and general physical condition to infection, and the fact that the previous history of these bodies as regards respiratory disease could be accurately ascertained for a period of time sufficiently long to permit of an estimate of the prevailing health of the troops as a whole, made this particular epidemic a natural experiment with unusually good controls.

Considerable interest attaches to the problem as to where and by what channels the various brigades acquired their infection. With regard to the first brigade to enter the post, the 5th F. A. Brigade, it is impossible to determine the source of the infection. The most probable source would seem to be the civilian population of Besancon, a city situated about thirty kilometers from the post and one to which the men were permitted to go on pass.

The source of infection in the case of the next brigade to enter the post, the 58th F. A. Brigade, seems fairly clear. The brigade undoubtedly acquired its infection as a result of moving into infected barracks, for the two regiments of the brigade which moved into the barrcks in the post began each to have an epidemic of influenza three and five days respectively after moving into the post, although these regiments had previously had no cases of influenza, while the other regiment, which did not enter the post, had no epidemic at all. On account of the short interval elapsing between the entry of the brigade and the onset of the epidemic, as well as the fact that the epidemic in the two regiments began almost simultaneously it would seem most likely that the first soldiers to be affected acquired their infection as a result of living in infected barracks rather than from carrier or incipient cases among the post personnel. Had the infection been acquired through the latter channels one would have expected a longer interval before the onset of the epidemic, for there was not much opportunity for direct contact between the

permanent post personnel and the members of the visiting brigades. On the whole, then, the evidence would seem to point to the view that the 58th Brigade acquired its infection as a result of moving into barracks that had previously been inhabited by an infected body of troops. If this view is correct, it must be assumed that the more immediate source of infection was the dust that had accumulated on the floors and bedding of the barracks and had been contaminated with the oral secretions of the men, and also that the virus of influenza can retain its virulence for several days under such conditions.

The source of infection in the case of the next brigade is not quite clear. It is quite true that this brigade (the 6th) had little or no respiratory disease prior to its entry into the post, and the fact that a period of a fortnight intervened between the entry of this brigade into the post and the beginning of the epidemic indicates that the brigade was not infected at the time of entry nor was it on the verge of an epidemic at that time. It must therefore have acquired its infection in the post itself. The next question that arises is: Was the infection acquired as a result of moving into infected barracks, the actual source being infected dust left behind by the predecessors, or was it acquired from carriers or incipient cases among the post personnel? It is almost impossible to decide this question conclusively as the evidence is conflicting.

In favor of the view that the infection was acquired directly from the barracks is the fact that those regiments (3rd and 78th F. A.), which moved into the southwestern group of barracks suffered most, and these particular barracks were the ones that had been occupied by the regiments of the previous brigade which had shown the largest number of cases. There were proportionately twice as many cases in the southwestern group as in the southeastern group, which was occupied by the other regiment of the brigade, the 11th Field Artillery.

Against the view that the infection was acquired from the barracks is the fact that the southwestern group of barracks was cleaned fairly thoroughly before the troops moved in and also the fact that a considerable interval of time (a fortnight) elapsed between the entry of the troops and the onset of the epidemic. Had the 6th Brigade acquired its infection as a result of moving into infected barracks, one would have expected the epidemic to commence at an earlier date.

The possibility exists that the infection was acquired by those soldiers of the 6th Brigade who participated in the cleansing of the barracks and by this means the disease was transmitted to others. The preliminary part of the cleansing process, the handling of the bedding, was a dusty affair and the infection may well have been acquired in this manner although there is no positive evidence on this point. The fatigue parties that did the work were changed daily so that in the course of several days there were several different groups exposed.

Again, the infection may have been acquired from the permanent post personnel. There were always cases occurring in the post organizations and

it may have been that carriers or incipient cases in these organizations served as intermediaries of infection for the incoming brigade. On the whole this view seems to explain the source of infection in the 6th Brigade as well as any. It would fit in with the longer interval of time elapsing between entry into the post and onset of the epidemic observed in the case of this brigade.

The origin of the epidemic in the last brigade to enter the post, the 156th Brigade, is not of particular interest as the brigade underwent its epidemic while billeted in neighboring towns and entry into the post apparently did not alter the course of the epidemic in the brigade in any manner.

The fact that during the Summer and Autumn of 1918 three brigades of approximately the same strength entered the post of A. P. O. 704 and were quartered there in succession, and that each underwent an epidemic of influenza which was more severe than that sustained by the preceding brigade would indicate that the virus of the disease became progressively more virulent as succeeding groups of troops were attacked. It has long been known that the virulence of bacteria may be increased by successive passage through susceptible animals. The epidemic at LeValdahon would seem to offer an instance of increased virulence acquired by the virus of influenza as a result of successive human passage. This assumption would explain the almost step-like increases in severity of the epidemic as observed in different brigades.

Against such an explanation of the increased severity of the individual "brigade epidemic" it might be urged that, as each brigade became infected in turn and moved away it left behind a greater amount of infected material and as a result each incoming brigade received in turn a larger dose of infecting material. However, this argument can be met with the fact that the brigade which suffered the most, the 6th, moved into barracks which had been scrubbed and were as clean as it was possible to make them under the existing conditions, and also that the cleansing process instituted was one calculated to remove at least the greatest amount of such infectious material if it were present. In view of the fact that such cleansing measures were instituted before the brigade moved in, one would certainly not have expected in that brigade a more severe epidemic than that sustained by the preceding brigade had the virulence of the infecting agent and the resistance of the troops remained the same. The evidence is strong, therefore, that in this particular epidemic we have a fairly clear-cut demonstration of increased virulence acquired by the virus of influenza as a result of successive passage through human hosts.

The chief lesson to be learned from the epidemic, from a military standpoint, is the great danger of moving noninfected bodies of troops into barracks in which there have previously been organizations affected with respiratory diseases in epidemic form. This is by no means a new lesson. In the second place the experience at LeValdahon shows that not even cleansing measures as thorough as it is possible to make them under good campaign conditions will suffice to prevent infection entirely. It would seem that better results could be obtained by a complete avoidance of infected barracks.

rather than by trusting solely to the simple measures of cleansing that are possible and practicable when dealing with large bodies of troops.

E. SUMMARY

1. During the period July 1st to Mar. 1st, 1918, a respiratory infection diagnosed as influenza occurred in epidemic form among United States troops located in or around the post of A. P. O. 704, American Expeditionary Forces, France, situated at the French Artillery Camp LeValdahon.

2. More than 3,000 cases occurred in this epidemic.

3. Approximately 12 per cent of these cases developed pulmonary complications, with a resultant case mortality rate of approximately 46 per cent.

4. The epidemic manifested itself in a series of successive outbreaks as different artillery brigades, hitherto unexposed to the disease, were in turn exposed.

5. These successive outbreaks tended to be progressively more severe both in character and extent. Evidence has been brought forward to indicate that this fact was due to increasing virulence acquired by the infecting agent.

LABORATORY METHODS

A NOTE ON THE EFFECT OF AMINO ACIDS ON THE GROWTH OF TUBERCLE BACILLI*

By Peter Masucci, Glenolden, Pa.

I. INTRODUCTORY

THIS experiment was undertaken in response to complaints from the Tuberculin Laboratory that for several months the growth of tubercle bacilli on glycerine bouillon was very scanty. The same experience was reported by several laboratories throughout the country. Owing to the change in the peptone used, the peptone was held as the cause of the limited growth. It is a well-known fact that amino acids are essential in starting the growth of bacteria. They include elements of the much more complex bacterial protein. Amino acids, therefore, are the ultimate foodstuffs from which bacteria derive their essential requirement of nitrogen.

Experience had shown that a medium rich in amino acids influenced to a marked degree the growth of B. diphtheriæ, although there was not as a rule a corresponding increase in toxin production. Since the tubercle bacillus is a very slow growing organism, it was thought possible that a glycerine bouillon enriched in amino acids would hasten the growth as well as increase it. Glycerine bouillon was prepared containing various amounts of amino acids, and the effect on the growth of the tubercle bacillus was carefully noted.

II. EXPERIMENTAL

Glycerine bouillon was prepared according to standard procedures for bacteriologic purposes. The meat juice was prepared by the cold infusion method. Five hundred grams of ground beef were infused in 1000 c.c. of water overnight. The material was filtered and the filtrate made up to 1000 c.c. The meat juice was then divided into two parts, to one (A), was added 2 per cent "Difco" peptone; to the other (B), 1.7 per cent "Difco" peptone plus 0.3 per cent "Aminoids" (Arlington Chemical Co.). The reaction of both after the addition of 5 per cent glycerine, was adjusted as nearly as possible to the same Ph^+. After sterilization a sample flask from each lot was analyzed. The solids, ash, and total nitrogen were determined by the methods of the A. O. A. C. Special precautions were taken in determining the solids to avoid the volatilization of glycerine. The bouillon was heated in a vacuum oven to a constant weight at 75° C. for 48 hours. Total phosphorus was determined

*From the Mulford Biological Laboratories, Glenolden, Pa.

by Sato's[1] colorimetric method. Protein nitrogen was found by saturating the bouillon with $ZnSO_4$ in a slightly acid solution, and a Kjeldahl determination was made on the precipitate. The amino acids were determined by the Van Slyke method.[2] Both lots of bouillon were adjusted to PH 7.5 electrometrically. The results of the analyses of the two lots are given in the following table in percentages:

TABLE I

	Solids	Ash	P_2O_5	N	N ppt. by $ZnSO_4$	N as amino acids
A.	9.22	1.05	0.116	0.348	0.110	0.098
B.	9.19	1.00	0.176	0.369	0.098	0.130

It will be seen by the analyses that Lot B is much richer in amino acids and total phosphorus than Lot A. Lot B has 31 per cent more amino acids and 50 per cent more phosphorus than Lot A. The larger amount of phosphorus in Lot B is perhaps as instrumental in influencing the growth of the

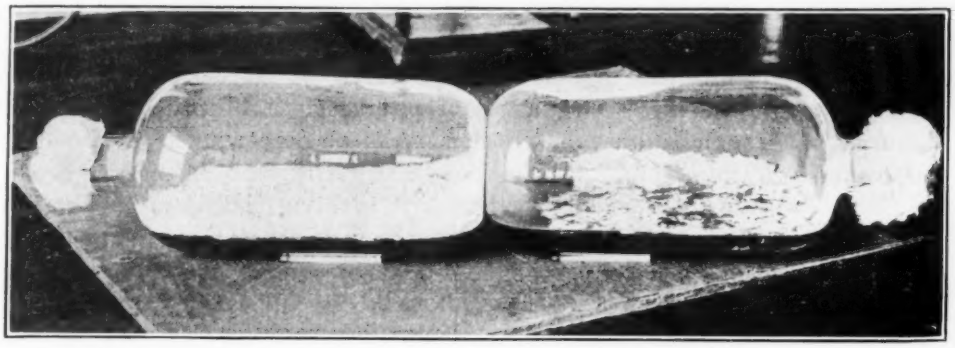


Fig. 1.

tubercle bacillus as the amino acids. This is made more evident when we consider the chemical constituents of the tubercle bacillus. This organism is rich in lipoids and especially in nucleo-proteins and lecithin proteins, both substances containing phosphorus in their molecule. It may be readily seen, therefore, that phosphorus plays an important part in their growth. Both lots of bouillon were planted with B. tuberculosis, human and bovine types, and incubated at 37° C. At the end of ten days, growth on Lot A was very scanty. Pellicle formation had not yet started. Lot B containing the aminoids, showed profuse growth with a complete surface pellicle. The amount of growth at this period was far greater than that of Lot A after six weeks of incubation. The bottle to the left (Fig. 1) shows the growth of Lot B and the one on the right that of Lot A. The difference in growth is very marked.

Several lots of glycerine bouillon were made containing 0.1 per cent to 1 per cent aminoids. The best growth seems to take place in bouillon containing 0.3 to 0.5 per cent aminoids. The bouillon now used in the Tuberculin Laboratory contains 1.7 per cent "Difco" peptone and 0.3 per cent aminoids, The results obtained are very satisfactory.

The function of glycerine in glycerine bouillon is not exactly known. Some writers claim that the tubercle bacillus does not utilize glycerine. However that may be, the osmotic pressure of Lot A and B was determined by the cryoscopic method. The results are given in the following table.

TABLE II

	Delta	Osmotic Pressure in Atmospheres
A.	2.610°	31.33
B.	2.580°	30.98

Ordinary plain bouillon has a freezing point depression of 0.6° to 0.9° C. or an osmotic pressure of 7.23 to 10.84 atmospheres. Whether glycerine plays any other function or not, one thing is certain, it increases the osmotic pressure of the bouillon threefold.

SUMMARY

Glycerine bouillon containing 1.7 per cent "Difco" peptone and 0.3 per cent aminoids has about 30 per cent more amino acids and 50 per cent more phosphorus than bouillon made with 2 per cent "Difco" peptone alone. The amino acids hasten the growth of B. tuberculosis by furnishing the necessary form of nitrogenous matter for the building of the more complex bacterial protein. The increased amount of phosphorus probably increases the quantity of growth as that latter element is an important constituent of nucleo and lecithin protein, substances found abundantly in the tubercle bacilli.

Whatever the function of glycerine is in glycerine bouillon, it is certain that the osmotic pressure of glycerine bouillon is three times greater than plain bouillon.

REFERENCES

[1]Sato, A.: Jour. Biol. Chem., 1918, xxxv, p. 473.
[2]Van Slyke: Jour. Biol. Chem., 1912, xii, p. 277.

"VARIATIONS IN THE WASSERMANN REACTION"—A REPLY*

BY ROBERT A. KILDUFFE, A.M., M.D., PITTSBURGH, PA.

IN the July, 1920, number of this Journal appeared a paper by Wilson† which, as tending, perhaps, to confuse the clinician depending upon the Wassermann test as a source of information, seems to invite some discussion.

Briefly, the paper emphasizes the following points:

1. That a cholesterinized antigen is more sensitive than an acetone-insoluble extract of beef heart.

2. That a cholesterinized extract may give a slight reaction with a normal serum.

3. That a positive Wassermann may be had in leprosy, frambesia, "hepatic disease," and scleroderma.

4. That blood taken after anesthesia may give a false positive reaction.

*From the Laboratories of The Pittsburgh Hospital, Pittsburgh, Pa.
†Wilson: Jour. Lab. and Clin. Med., July, 1920, v, No. 10, 670.

5. That serums should be collected in sterile tubes and examined as soon after collection as possible.

6. That "we have repeatedly demonstrated than anticomplementary substances develop in serums if kept for a considerable time, which not only inhibit hemolysis in antigen and control tubes, but certain substances are formed, presumably by bacterial growth or action on some substances in the blood serum, creating an inhibition of hemolysis in the antigen tubes alone, causing, perhaps, a negative serum to become positive."

7. That care should be taken in interpreting the significance of "weakly positive up to two-plus reactions" in cases giving a negative history and without symptoms of syphilis.

8. That reactions of varying degree may be obtained with different antigens and the same serum.

Many of the above statements are, of course, merely repetitions of facts well known to serologists in general and embody nothing new.

That cholesterinized extracts must be controlled by less sensitive antigens is so well realized that it is safe to say that no competent serologist, now-a-days, would attempt a complement-fixation test with less than two antigens—a cholesterinized extract and an acetone extract—the writer using, in addition, a plain alcoholic extract of either normal beef or human heart, or luetic liver.

Moreover, no antigen of any kind should be used whose full anticomplementary and antigenic dose is not known, and which has not been tested, along with well-known and tested antigens, in at least from twenty to fifty tests to ascertain that its reactions are consistent and reliable. In addition, knowing that antigens may rapidly and suddenly deteriorate, all antigens should be re-titrated at monthly intervals.

If these conditions are fulfilled, fewer inconsistent and inconstant reactions will be obtained.

To one accustomed to using a triple antigen battery, the varying strength of reactions obtained with varying antigens is well known. It has not been my experience, however, that the untreated, four-plus case gives anything but four-plus with all three. In the treated case, the varying reactions with varying antigens is well-illustrated and can be utilized as a means of following the efficacy of the treatment, as the reaction first disappears in the plain extracts, then in the acetone extract, and lastly in the cholesterinized extract which may disappear very slowly.

In regard to the nonspecific, positive reactions obtained with cholesterinized extracts: while this may be taken as the attitude of serologists in general, time alone will show if this error is as marked as it is said to be, and, in any event, the error is controlled by the use of multiple, less sensitive antigens.

The question resolves itself, as has been stated time and time again, into one of the *interpretation* of the test, and not the test alone, a matter which I have discussed elsewhere.*

In the unknown case, submitted for diagnosis, one would hesitate, in the presence of a negative reaction with all but the cholesterinized extract, to

*Kilduffe: The Practical Value and Utilization of the Wassermann Test in General Practice, Arch. Diagnosis, Jan., 1920.

forthwith diagnose syphilis. Experience, however, would indicate the advisability of a repetition of the test after a short course of antiluetic treatment, in other words, a "provocative Wassermann."

With the same reaction in a patient under treatment, or where the history is to be had, such a reaction can justly be interpreted as indicating the necessity for further treatment.

In the weakly positive case without history or symptoms, it is a simple matter to repeat the test with another triple antigen battery, or, better still, to do a provocative Wassermann which is likely to be plainly positive. That a positive Wassermann may occur, in the absence of syphilitic infection, in leprosy and yaws is well known. Yaws has, for its etiologic agent a spirocheta, (Spirochetæ pertenuis) which is almost indistinguishable, morphologically, from Treponema pallidum—a fact which may account for the occurrence of the reaction. In any event, neither leprosy nor yaws should be mistaken for syphilis, so that this fact does not vitiate the reliability of the Wassermann.

That a positive Wassermann, in the absence of syphilis, may also be had in "hepatic diseases" in general, is, perhaps, not so universally admitted; and that positive reactions obtained in "hepatic diseases" are always obtained in the absence of syphilis, is, perhaps, debatable.

The question of positive reactions in scleroderma cannot be given a marked importance as affecting the reliability of the test, for, occasionally, syphilis and scleroderma are co-existent and, when this is not the case, the condition is one not easily confused with syphilis. The cause of scleroderma being entirely unknown, it is not beyond the bounds of probability that the positive reactions may arise from the same combination of circumstances responsible for them in frambesia—the presence of a spirocheta closely related to the Treponema pallidum.

The knowledge of the necessity of sterile glassware and of collecting and keeping serums under aseptic conditions goes back to the days of the first description of the test, and the necessity for avoiding bacterial contamination is, likewise, well known.

It is with the last statement in the paper that this reply is principally concerned. If it is true that "bacterial contamination and bacterial action on some substance in the blood serum," will create "an inhibition of hemolysis in the antigen tubes alone, thus causing a negative serum, perhaps, to become positive"—this statement, though justly qualified by "perhaps," should arouse much interest. Certainly, the exact methods whereby such a conclusion could be reached should be known, so that the observation may be repeated and confirmed by others.

Personally, the writer feels that a complement-fixation test properly performed, with antigens whose anticomplementary and antigenic range is definitely known, and in the presence of adequate controls, *when properly interpreted,* constitutes a safe and reliable guide to the diagnosis of syphilitic infection.

The absence of a history is not always to be relied upon; the absence of symptoms readily detectable, is a phenomenon of frequent occurrence in this

protean disease; and the conditions other than syphilis in which a positive fixation may occur are too morphologically distinct to be a justifiable cause for confusion.

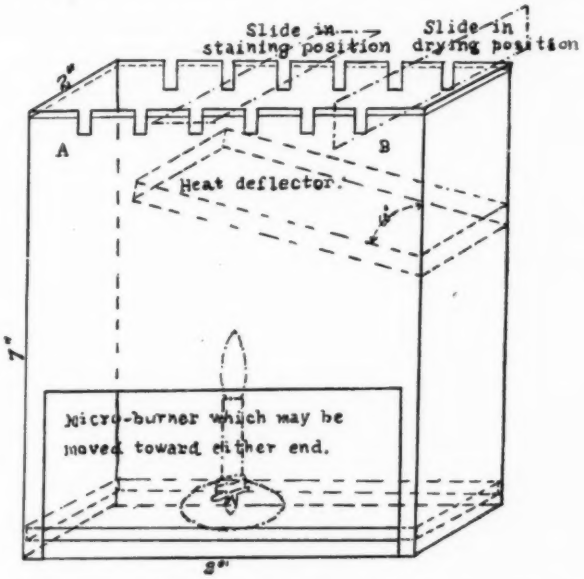
That the reaction has its limits of delicacy is, of course, well known, and it is to be hoped that there are few clinicians, and still fewer serologists who would place absolute dependence upon a single negative Wassermann as indicating absolute freedom from syphilitic infection; conversely, the occurrence of a positive below the four-plus variety, is to be always intelligently interpreted in terms of its relation to the patient and his condition.

APPARATUS FOR STAINING AND DRYING SLIDES

By Frederick H. Lamb, M.D., Davenport, Iowa

THE accompanying diagram represents an apparatus for staining and drying microscopic slides, which I have used in laboratory work for five or six years. It is easily made by any copper-smith, inexpensive and has proved to be very satisfactory.

The apparatus consists simply of an oblong box, made of sheet copper, preferably, although galvanized sheet iron will serve the purpose and is less expensive. The top of the box as it stands in the diagram is open. Along



each side and directly opposite to each other, there are small slots 1/8″ wide and 1/2″ deep spaced 1″ apart, in which slides may be placed to drain and dry. The bottom of the box consists of a piece of sheet copper with flanged edges and placed as shown in the diagram. This serves as a stand or base for a micro-burner. One side of the box is cut out as shown, so that the burner may be moved from one end to the other, depending on the temperature which is desired under the slides. About half way between the bottom

and top of the box there is a piece of copper sheeting with edges flanged and inclined as shown in the sketch. This acts as a heat deflector. It does not extend across the entire width of the box, but stops short of one end at about 1½ inches, so that if necessary heat from the burner may pass directly up to the slide. The over-all dimensions are: 8″ wide, 7″ high and 2″ deep. The width may be increased an inch or two to accommodate more slides if so desired.

The apparatus is especially useful in staining with carbol-fuchsin for tubercle bacilli. The slide may be placed in the position "A" and with the microburner in position "X," there will be just enough heat to cause the stain to steam. With a slide in position "B" the temperature is about 10° C, above room temperature.

Aside from its direct usefulness, the apparatus affords a safe and clean way of handling infectious material.

BLOOD CHANGES IN A CASE OF HEMOPHILIA AFTER TRANSFUSION*

By Harold A. Bulger, M.D., Boston, Mass.

ALTHOUGH the use of Blood serum and blood transfusion in hemorrhagic diseases is not an uncommon procedure, there are few instances on record in which even the effect on the coagulation time has been described. It is of interest, therefore, to report the following study of the changes in the factors of coagulation in a case of hemophilia.

It is well recognized that the results of transfusion in hemophilia are temporarily beneficial even if they are not permanent. How lasting the results are is not definitely known. Information on this question would be of value as indicating whether the degree of change is great enough to allow operative procedure.

Minot and Lee[1] report a case with coagulation time of 150 minutes. After transfusion of 600 c.c. of human blood (method not stated) the coagulation time was normal, but in three days it was 60 minutes and in five days 100 minutes. Addis[2] reports two cases. In one, injected intravenously with 15 c.c. of human serum, the coagulation time was 62 minutes before and 24 minutes after injection. After twelve days it had risen to 127 minutes but in three weeks had fallen to 86 minutes. The second case was transfused with 300 c.c. of phosphated blood. Before transfusion the coagulation time was 245 minutes; after transfusion 24 minutes. After twenty-five days the coagulation time was 200 minutes and at that time 8 c.c. of human serum injected intravenously brought it down to 38 minutes.

A boy (R. E. W., Med. No. 11486), age 14 years, with a typical family history of hemophilia, had had attacks of swelling in the joints and at one time

*From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.
This is study No. 8 of a series of studies on the physiology and pathology of the blood from Harvard Medical School and allied hospitals.

very serious bleeding after the extraction of a tooth. For the past five years he had repeated attacks of hematuria averaging about two weeks in duration. Three weeks before admission, following whooping cough, blood appeared in the urine again, and the night before admission his left knee became greatly swollen. During his stay in the hospital the hematuria gradually disappeared but the other knee became swollen and later both elbows became involved. He was transfused by Dr. Cunningham with 300 c.c. of his mother's blood by the citrate method, and following this the swelling gradually subsided. Eleven days after transfusion his right elbow and ankle both showed fluctuant swelling again but they rapidly subsided. Hematuria did not recur.

Just before transfusion and at various intervals following, blood was obtained by veni-puncture in a glass syringe rinsed in salt solution. Part of the blood was used to determine the coagulation time while the remainder was oxalated and the oxalated plasma separated. On most occasions a normal control

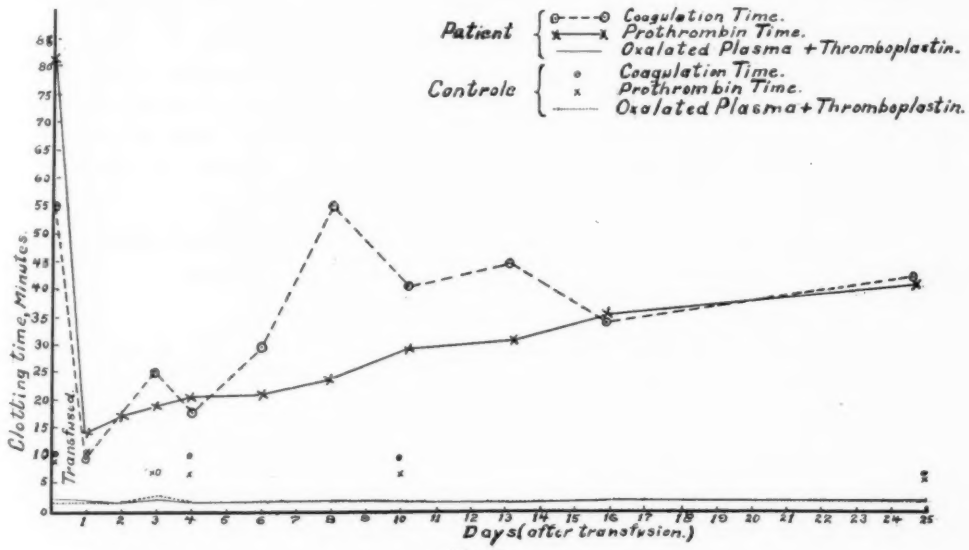


Fig. 1.

specimen was obtained in a like manner. With each specimen of oxalated plasma the "so-called" prothrombin time was determined by the method described by Howell and also the effect of adding thromboplastin solution on the coagulation of the oxalated plasma. The changes are shown by the accompanying graphs.

COMMENT

It will be noted that the day following transfusion the blood was practically the same as the control, but that the coagulation time and prothrombin time gradually lengthened. One month later they were still less than before transfusion. There was no marked change in the blood found to correspond to the slight bleeding into the joints found on the eleventh day.

The effect of thromboplastin on the clotting of the oxalated plasma is of interest in relation to the cause of hemophilia. It has been suggested that hemophilia is due to insufficient prothrombin in the blood, but in this case the addition of thromboplastin to the oxalated plasma caused it to coagulate

as well as the normal control. This suggests that there was sufficient prothrombin and that thromboplastin was lacking.

REFERENCES

[1]Minot and Lee: Arch. Int. Med., 1916, xviii, 1.
[2]Addis: Proc. Soc. Exper. Biol. and Med., 1916, xiv, 19.

A SIMPLE METHOD OF ISOLATING BACTERIA FROM PATHOLOGIC MATERIAL*

By H. C. Klix, Detroit, Mich.

THE object of this paper is to present a simple and rapid method for the isolation of bacteria from pathologic material. It has been used by the writer for a number of years in the routine examination of mixed cultures, pus, sputum, and other material submitted for the preparation of autogenous vaccines. It offers a number of advantages over the ordinary plating method.

The use of Petri dishes is not only cumbersome, but is unadapted to certain culture media, particularly those containing unheated body fluids. The exposure incident to handling plates also increases the danger of contamination. The following method overcomes these disadvantages, is simple of execution, and can be satisfactorily applied to practically all bacteriologic examinations.

EQUIPMENT

A few tubes of suitable culture media, usually ascites agar slants, a small tube of bouillon, and a platinum loop.

TECHNIC

One-fourth c.c. of bouillon or water of condensation is pipetted into each of four tubes of solid media and the tubes numbered I, II, III, and IV. A small amount of pathologic material is transferred to Tube I and mixed by means of platinum wire with liquid (bouillon or water of condensation). Two or three loopfuls of the bouillon in Tube I are transferred to Tube II and mixed as before. Two loopfuls of bouillon from Tube II are transferred to Tube III. One loopful from Tube III is transferred to Tube IV.

Tubes are shaken to break up any clumps of original material or bacteria, and bouillon is allowed to run over the entire surface of the slant by properly inclining the tube. Tubes are placed in rack in upright position and transferred to incubator. After 24 hours' incubation isolated colonies are picked up by means of platinum wire and transferred to individual tubes of culture media.

Somewhere in the series nicely isolated colonies will be found which can be readily picked up.

The chief advantage of this method aside from its simplicity is its adaptability to any kind of culture media.

*From the Research Laboratories, Parke, Davis & Co., Detroit, Mich.

The Journal of Laboratory and Clinical Medicine

VOL. VI. NOVEMBER, 1920 No. 2

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Ann Arbor, Mich.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
HANS ZINSSER, M.D. - - - NEW YORK
PAUL G. WOOLLEY, M.D. - - DETROIT
FREDERICK P. GAY, M.D. - - BERKELEY, CAL.
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
WARREN T. VAUGHAN, M.D. - . BOSTON

Contents of this Journal Copyright, 1920, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

Influenza and Tuberculosis

FOLLOWING the 1918 and 1920 epidemics of influenza, there has arisen in the literature some controversy regarding the effect, if any, of influenza on tuberculous individuals. This has centered particularly on the question whether tuberculosis produces some degree of immunity to influenza, and whether the latter on the other hand predisposes either to the lighting up of a latent tuberculosis, or to a new infection with the tubercle bacillus. Keen observers in the field of tuberculosis who have had apparently equal opportunities to study the effects of the pandemic differ radically in their conclusions.

After the 1889-1893 epidemics, Leichtenstern recorded that the mortality tables of all countries agree in showing considerable rise in the mortality from pulmonary tuberculosis in influenza periods. The clinicians of that time made the frequent observation that the course of tuberculosis in the lungs is markedly and unfavorably influenced by grip and its pneumonic complications. Latent quiescent cases often became active, and healed and healing foci broke out anew. Afebrile cases were changed to the hectic type and frequently hemoptysis was induced. In London, during the height of

the 1889 epidemic, the weekly death reports from phthisis rose to double the average. The increase in death rate during the epidemic period was not limited entirely to tuberculosis, but there was almost a doubling of deaths due to all acute respiratory infections. After the cessation of the epidemic, however, there was some decrease in the general mortality, as well as in the mortality from respiratory infections. This was especially true of deaths from pulmonary tuberculosis, which decreased to such an extent that the total mortality rate for the year for this disease was little greater than for preceding years.

Similar observations have been made following the 1918 pandemic. Jordan remarks that in New York City in 1918 during the two weeks of maximum epidemic mortality, the deaths reported from pulmonary tuberculosis numbered 430, as compared with 264 for the corresponding weeks of 1917. Vaughan and Palmer found that the deaths from tuberculosis in the army were higher in the autumn of 1918 than in the two previous four months' periods, the death rate rising from 18 per 100,000 during the summer to 46 per 100,000 in the autumn. The rate for the same time of the preceding year had been 15 per 100,000. They assume that the most plausible explanation for this increase in deaths is that dormant and incipient cases introduced into the army during the preceding year had accumulated and possibly were hastened into the acute stage, both by the duties of camp life, and the prevalence of the epidemic of grip and pneumonia. Quite naturally there have been from the time of the first assembling of troops an accumulation of tuberculous individuals, inasmuch as such men were not discharged, but were kept in the army and under Government control and supervision. Sir Arthur Newsholme in reviewing the relationship between influenza and tuberculosis in England remarked that so far as the national statistics in that country are concerned, every year showing an extensive death rate from the former has been followed by a year in which the death rate from the latter has been excessive. He concludes that many deaths from tuberculosis are undoubtedly hastened during an influenza epidemic. Abbott wrote of the epidemic of 1889 in Massachusetts that the chief diseases which followed in its train and were intimately associated with it were bronchitis and pneumonia, and that phthisis when already existing in the victim of the attack was undoubtedly aggravated and in many cases a fatal termination was hastened. Baldwin says that influenza is a frequent and important agent in bringing latent tuberculosis to life. "Allowing for mistakes in diagnosis in which the presence of tuberculosis is overlooked, influenza must be classed as an important exciting cause, if not a true predisposition."

In frank opposition to the foregoing authorities, Fishberg claims that influenza has had no effect whatever on the course of tuberculosis. He says that a large proportion of tuberculous patients under treatment in New York City in 1918-1919 contracted the disease and not a single one succumbed. This appears as rather an inclusive statement. He goes on to say that some were in the far advanced stages of the disease, with large cavities in the lungs, and yet they passed through the acute symptoms and recovered, the tuberculous process then pursuing its course as if no complicating disease

had affected them. He believes that the prognosis was if anything better in those who suffered from tuberculosis or any other chronic pulmonary disease, such as asthma, bronchitis, emphysema, bronchiectasis, than in those in whom the lungs and bronchi had been apparently in healthy condition. Fishberg observes that, instead of lighting up the tuberculosis, the influenza runs a milder course than when attacking healthy persons, and the old lung lesion remains in about the same condition as could be expected if no complicating process had attacked the patient. He says that authors who have asserted the contrary have based their arguments mainly on the fact that many tuberculous patients date the onset of their tuberculosis as concurrent with an attack of influenza; that many patients suffering from phthisis state that ever since an intercurrent attack of influenza the symptoms of tuberculosis have become more pronounced; that the Pfeiffer bacillus has been found quite frequently in the sputum of tuberculous patients, especially that derived from pulmonary cavities; and that in some countries it has been noted that during and soon after an epidemic of influenza the mortality from tuberculosis was increased.

He believes that many of the conditions diagnosed as influenza have been no more than ordinary colds and that the average patient will call any upper respiratory tract infection grip during or around the time of an epidemic. He further believes that a misdiagnosis of tuberculosis is frequently made in influenza convalescents who show some signs of moisture in their lungs which does not clear up for some time, causing doubt in the mind of the examiner but which is not truly tuberculous in origin. Fishberg cites P. J. Murphy, Hawes, Armstrong, McRae, and Dickinson, as well as Geiber and Schlesinger in Vienna, and Rickmann and Ladeck in Germany, as having observed the same phenomenon of relative insusceptibility of tuberculous patients and failure of influenza to hasten the progress of tuberculosis. He also calls attention to the low incidence of influenza in tuberculosis sanatariums, but apparently compares this incidence with the incidence for the public at large, and not with that in similar institutions devoted to the care of invalids with diseases other than tuberculosis, or with other institutions in general.

Amberson and Peters, as well as Minor, take sharp exception to the statement of Fishberg and the former have collected the evidence against Fishberg's view. They first point out that a comparison of the incidence of 5.4 per cent among hospitalized tuberculous patients at Chicago cannot be compared with a much higher incidence of the epidemic in the various military camps. As Heiser has pointed out, the mere quartering of men in barracks seems to have a tendency to increase the risk from acute respiratory diseases. Furthermore, the incidence at some sanatoria was low, while at others it was high, nearly as high as for the community at large. In Hawes' report of the epidemic among the Massachusetts sanatoria, Lakeville had escaped entirely, while Rutland which consisted chiefly of ambulatory cases less easily controlled, had an influenza incidence of 18.3 per cent among the patients, and 21.3 per cent among the employes. At Montefiore Home, the proportion of tuberculous patients and employes contracting the infection was practically the same as among the nontuberculous employes, and about

BARLOW MEDICAL LIBRARY
742 NORTH BROADWAY
LOS ANGELES, CAL.
LIBRARY OF THE
LOS ANGELES COUNTY MEDICAL ASSOCIA
534 SOUTH WESTLAKE AVE.

the same percentage of both groups developed evidence of broncho-pneumonia.

Still another fallacy in the comparison of incidence in institutions and the like is proved by the work done by Jordan, Reed and Fink, who found that in the various Chicago telephone exchanges the attack rate varied from 5 per cent to 27 per cent, although the working conditions were approximately the same. The attack rate in one section of the students army training corps in Chicago was 3.9 per cent, while in another section particularly exposed to infection it was 39.8 per cent. Similarly Frost found the incidence in Louisville, Kentucky, to be 15 per cent, and in San Antonio, Texas, 53.3 per cent. All of these figures show the difficulty of comparing rates for various institutions and various groups of individuals. Although Fishberg quoted Rickmann in support of his contention that influenza has no effect whatever upon tuberculosis, Amberson and Peters used his work in support of their contention, and call attention to the fact that in 30 out of 40 tuberculous persons reported by him who had contracted the grip, the attack did not produce any aggravation of the lung condition. Presumably it did in the other ten. If even 25 per cent of tuberculous patients who contract influenza have their pulmonary condition aggravated, this should be regarded as a notable number. According to Stivelman, 11.4 per cent of tuberculous influenza cases died at Montefiore Home. In a survey of convalescents from the Loomis Sanatorium, Amberson and Peters found that 70 had contracted influenza, or 5.7 per cent of the number surveyed, and that 11.4 per cent of these had had relapses of their pulmonary condition, apparently due to the acute disease, while 22.9 per cent had died from the intercurrent infection. Two and eight-tenths per cent were deaths due to tuberculosis after convalescence from the influenza. Tubercle bacilli have been found in the sputa of convalescent grip patients, whose sputa had previously been negative, by Amberson and Peters, as well as by Berghoff, at Camp Grant. The latter found that 50 per cent of his cases showed a reactivation and a positive sputum after an attack of influenza.

Amberson and Peters agree with Fishberg in the observation that there has been no increase in the general mortality from tuberculosis within the recent months, and suggest as an explanation the possibility that during the epidemic enough of the old cases were carried off to account for a temporary lull until new cases developed, or others had time to reach later stages of the disease. As we have previously remarked, Leichtenstern observed this same phenomenon following the 1889-1890 epidemic.

The state of our knowledge of influenza and tuberculosis is considerably clouded by divergent opinions such as those quoted above. To further complicate the picture, there are other authors who assume a middle ground and contend that there is some truth in both lines of contention. Thus, Amelung believes that the morbidity among patients with pulmonary tuberculosis is slight, and that the grip takes a milder course in such patients than in the nontuberculous unless the disease is far advanced, but that pulmonary tuberculosis may and sometimes does follow the disease in patients whose lungs

were previously sound, and that in the last-mentioned cases the prognosis is relatively bad. Peck finds that in some tuberculous patients the disease has been aggravated, but in the majority the intercurrent influenza did not appear to have been the causative factor in the acute exacerbation of the tuberculosis.

Debré and Jacquet have reviewed the European literature on the subject pro and con, and though they admit that there are exceptions, as at l'hôpital Tenon, where, in a barracks reserved entirely for female tuberculosis patients there was a veritable epidemic of grip, 29 per cent of the 28 being attacked in a few days; and at the sanatorium de La Tronche, where 83 per cent took ill between the 25th of September and the 20th of October; that as a rule tuberculous individuals are less heavily attacked by the influenza than are the nontuberculous. As they suggest, the first explanation that comes to mind is that the tuberculous are isolated in the hospitals where general hygenic conditions are good, but we have all seen other institutions, hospitals, etc., in which the inmates were not spared as they were in tuberculosis hospitals. Furthermore, in certain sanatoria, such as the sanatorium of the Cote Saint-André, and Bligny, and several German sanatoria, the proportion of tuberculous individuals attacked was very much less than that of the professional attendants, the physicians and nurses. Again, where cases have occurred in these hospitals, and little precaution was taken to prevent its spread, very few other individuals took sick. Finally, many have noted the infrequency of the disease even in those tuberculous individuals who were living at home. It has been suggested that rest in bed from the beginning of the attack explained the mildness, or that the immunity resulting from the infection with pneumococcus, streptococcus, etc., in tuberculous individuals explained the absence of pulmonary complications. Marfan, who observed this same phenomenon in 1890, suggested that it might be due to a refractory state of the tubercle bacillus against the virus of influenza. Debré and Jacquet conclude that none of these explanations is satisfactory.

Having concluded that tuberculosis does protect in some measure against influenza, Debré and Jacquet next discuss whether the latter has increased the severity of tuberculosis in the subjects who were already tuberculous. They review the literature and make their conclusions, not from statistical records, but from general observations. They consider first those cases of phthisis which are open cases when attacked, and second, latent tuberculosis. Their conclusion concerning the first group is that influenza does not have any effect on the rapidity of evolution of the tuberculous process, except in very rare instances, such as an occasional case of miliary tuberculosis following grip. As regards latent tuberculosis, however, they do believe that the intercurrent acute infection does cause in many cases a lighting up of a previously entirely dormant tuberculosis. It seems rather difficult to reconcile the two ideas. If one type of tuberculous individual is rendered more susceptible to the ravages of consumption, it would seem reasonable to expect that all types would be so affected.

The greatest difficulty in reaching a conclusion regarding the effects of

influenza on tuberculosis, and vice versa, is due to the fact that the individuals studied are in all stages of the disease, and that each individual reacts differently and in his own way. Opinions have been based chiefly on clinical observations, and not on statistical study of large series of cases, while from the nature of the conditions, even statistical studies would not be without great fallacy.

Armstrong, who has made as nearly a complete statistical study as is possible under the circumstances found that in Framingham, Massachusetts, 16 per cent of the entire population was affected with influenza, but only 4 per cent of the tuberculous group in the community was so affected. Most of these latter were of the arrested type and were going about the community taking their part in industry and exposed to the same degree of contact as was the case with the normal population. The fatality rate was equally in contrast. Armstrong concluded that there appeared to be a relative degree of protection for the highly tuberculized. If we accept these figures at their face value we must conclude then either that tuberculosis offers some degree of protection against acute influenzal affection, or, that the tuberculous of Framingham have been so well trained in sanitation and personal hygiene, as a result of the Framingham demonstration, that they have been able to protect themselves against the grip. In the latter case we must look upon the result as a successful demonstration of the principles of preventive medicine. Certainly this did play a part, to the extent at least that individuals knowing themselves to be infected with tuberculosis, and knowing themselves to be in the presence of a pandemic, became more wary of crowd contact, and in case they did become ill, they undoubtedly went to bed at the earliest opportunity.

If, on the other hand, this is a true demonstration of relative immunity in a chronically infected individual, the explanation must be sought elsewhere. Does a chronic respiratory infection confer a relative degree of immunity to an acute respiratory disease? Do the germs already on the premises exert, so to speak, "squatters' rights?" Are we observing an example of non-specific immunity due to local preceding infection? Still another factor must play an important role, the factor of race stock. The excess of tuberculosis in negroes, for instance, over that in whites, is in some localities double or treble, while various observers, as Frost, Brewer, and Frankel and Dublin, report that the influenza incidence and mortality among negroes was decidedly less than that among the whites. Winslow and Rogers found that in Connecticut the proportion of influenza-pneumonia deaths is lower than would be expected among persons of native Irish, English and German stock, and higher than was to be expected among Russian, Austrian, Canadian and Polish stock, while it was enormously high among the Italian. Italians are notably insusceptible to tuberculosis, while the Irish are much more prone to infection with the disease. For instance, in Framingham, where the tuberculosis incidence rate for the entire population was 2.16 per cent, the rate in the Italian race stock was .58 per cent, and in the Irish 4.80 per cent. In Framingham there was about four times as much influenza among the Italians as among the Irish. Is this apparent insusceptibility of certain race stocks an inherent condition, or is it dependent chiefly on differences in living

conditions and in age prevalence in the different races? Probably it is chiefly the former. Frost, for instance, found that among the negroes the incidence of influenza was lower even though the living conditions were much poorer than those among the whites.

Armstrong's survey has also thrown some light on the effect of the influenza on previously tubercularized individuals. In a survey of 700 individuals who had had the acute disease there were ten arrested cases of tuberculosis, or 1.4 per cent. All these had been known to be arrested cases previous to the epidemic, and in none of them did the disease appear to have been actively and permanently lighted up. Some had manifested a slight activity, but all seemed to be on the way to a rearrest of the disease. On the other hand, 13 cases, or a 2 per cent of the 700, were found to have active tuberculosis which had hitherto been undiagnosed, and an additional 8 cases, with indefinite bronchopulmonary signs, were designated as incipient tuberculosis cases. This is to be contrasted with an incidence of active tuberculosis in the preepidemic examination of approximately 1 per cent. These figures would indicate an increase in tuberculosis incidence. How may this be explained?

It has long been known that individuals with measles will not react to tuberculin tests, even though they have been positive before developing the measles, and though they will become positive again after recovery. The same may be said of vaccination. Individuals vaccinated against smallpox, who have measles, and are during their illness revaccinated, will not show an immediate reaction. The test will remain entirely negative, while after recovery, the immediate reaction may be obtained. Normally, it will appear in 95 per cent of cases, while among those with measles the phenomenon remains absent in 90 per cent. The same may be said of certain other acute illnesses, particularly scarlet fever. The phenomenon has been variously explained. Von Pirquet, who was the first to observe it in measles, believed that the acute disease created a temporary inability to produce antibodies, and therefore designated the condition by the name anergie. The same phenomenon of anergie has been found recently to hold in the case of influenza. Debré and Jacquet, Lereboullet, Bloomfield and Mateer, as well as Berliner and Schiffer, have brought abundant evidence to this effect following the 1918 pandemic. It has also been shown by Cayrel and others that there is a diminution of typhoid agglutinins in the serum of influenza patients vaccinated against typhoid. The agglutinin titer again increases after recovery. It is true that agglutinin titer is not a measure of immunity, but it is frequently used as such and serves to give us some information on the subject. If, then, influenza is an anergie disease, a "maladie anergisante," we have a theoretical explanation of the increase in severity of tuberculosis following the acute infection. We have long known that measles predisposes to tuberculosis. We have recently been thoroughly convinced that influenza lessens resistance to secondary infection with streptococcus, pneumococcus, and other respiratory tract organisms. Shall the tubercle bacillus be added to this list? During the 1918 epidemic we saw men in the army camps who passed through an attack of influenza-pneumonia and died within a few weeks from tuberculous pneumonia or miliary tuberculosis. These men had previously been so free

from signs of their tuberculosis as to be accepted for military service as healthy individuals. The number of these cases was small, to be sure, but sufficiently large to convince us that there do exist instances in which tuberculosis is tremendously fired by an intercurrent influenza.

If we may judge merely by the balance of evidence and risk any conclusions from such conflicting testimony, we may sum up as follows:

1. Great variation in the interaction of tuberculosis and influenza must be expected, because of the many stages at which the tuberculous may be attacked, because of the altered mode of living of known consumptives, and because of the protected life of most of them.

2. Phthisical patients as a group, are *relatively* insusceptible to influenza infection. This may be due to the tuberculous process itself or to some extrinsic but nearly related cause.

3. But many individuals with pulmonary tuberculosis *do* get influenza.

4. And the disease, having been contracted, in many cases hastens the fatal termination of the tuberculous process.

5. It may be that this phthisical exacerbation occurs more frequently in individuals with latent tuberculosis, individuals who are not at the time mobilizing their protective antibodies.

BIBLIOGRAPHY

Leichtenstern, O.: Nothnagel's Specielle Pathologie und Therapie, 1896.
Jordan, E. O.: Proc. Inst. Med. (Chicago) 1918-1919, ii, 135.
Vaughan, V. C., and Palmer, Geo. T.: Jour. Lab. and Clin. Med., 1919, iv, 586.
Newsholme, Sir A.: Jour. Am. Med. Assn., 1919, lxxiii, 890.
Abbott, Samuel W.: Twenty-first Annual Report of State Board of Health of Mass., (Pub. Doc. No. 34) 1890, pp. 307-384.
Fishberg, M.: Am. Rev. Tuberculosis 1919, iii, 532.
Murphy, T. J.: Boston Med. and Surg. Jour., 1919, clxxxi, 266.
Hawes, J. B.: Boston Med. and Surg. Jour., 1919, clxxx, 35.
Armstrong, D. B.: Boston Med. and Surg. Jour., 1919, clxxx, 65; Am. Jour. Pub. Health, 1919, ix, 960.
MacRae, Duncan M.: Lancet, London, 1919, i, 281.
Dickinson, W. H.: Lancet, London, 1919, i, 314.
Amberson, J. B., Jr., Peters, A., Jr.: Am. Rev. Tuberculosis, 1919, iii, 359; ibid, 1920, iv, 71.
Jordan, E. O.; Reed, D. B.; Fink, E. B.: U. S. Public Health Reports, xxxiv, 1528.
Frost, W. H.: Public Health Reports, Mar. 12, 1920, xxxv, No. 11, p. 584.
Frankel and Dublin: Am. Jour. Pub. Health, 1919, ix, 731.
Stivelman, B.: New York Med. Jour., 1919, lx, 20.
Debré, Robert and Jacquet, Paul: Paris Médical, 1920, p. 24.

—*W. T. V.*

What We Know and Do Not Know about Tuberculosis

IT appears to be a law of our still rudimentary intelligence, that the less we know of a subject the more dogmatic we are inclined to be about it. This human trait, no doubt a part of the strategy of self-defense, is largely responsible for the perpetuation of ignorance and error. The mind which can free itself from prejudice and foregone conclusions, from assumption and assertion, is the mind which discovers new realities.

In nearly all departments of what we call medical science, our knowledge is so fractional that the term science has a flavor of satire; yet how con-

fidently we make our own sweeping unproved assertions, and how hotly we contradict each others'.

Opinion aside, what do we positively know about, for example, influenza, malignant disease, the complement deviation tests, vaccines, common colds, tuberculosis?

We have tried to analyze the status of this last subject, tuberculosis, from the point of view of the things we know—the things, at least, that we are agreed on at present—and the things we do not know, that is to say the things about which we disagree and debate. It is, of course, impossible to tabulate all we do not know: we have included only some of the more important points which call for solution.

We know:

That tuberculosis is infectious, parasitic in origin.

That it is caused by a specific organism, the tubercle bacillus.

That certain more or less characteristic tissue changes occur in its course.

That the conflict between bacillus and host often ends very early in favor of the host—so early that the presence of lesions is not suspected during life.

That in the majority of cases which are recognized clinically, the disease shortens life.

That its morbidity and mortality rates are high throughout the civilized world.

That there is a group of symptoms and signs, some or all of which occur in recognized cases.

That exhaustion and malnutrition in the host contribute to the development of the disease, while rest and adequate feeding favor recovery.

We do not know, or do not know thoroughly:

The biology of the parasite, how simple or complex, constant or variable, is its life cycle.

The significance of variations in the parasite, as the human and bovine strains, and their relation to each other and to the production of disease in various hosts.

The explanation of virulence and resistance, and the relative importance of these factors and of dosage in the development of disease.

The role of family and racial heredity, and of home exposure.

The relative importance of various modes of exposure and portals of infection.

The truth about such alleged predisposing factors as influenza and children's diseases.

The importance of carrier cases.

The physiology of defense, and the part played in it by body fluids and by special cells (e. g., endothelial leucocytes), tissues (e. g., lymphoid), and organs (e. g. adrenal).

The absolute and relative frequency of healed and active tubercle, and the amount of variation in these figures in different localities and classes, and under different conditions of housing, etc.

The occurrence of healed tubercle among savage races to whom tuberculosis is a new disease.

Whether infection, with invasion and some response on the part of the tissues, is practically universal in civilized races, whether it usually occurs in childhood, and whether an active immunity is commonly the result.

The effect of other diseases and of mental states on tuberculosis, and vice versa.

The effect of tuberculin, and the indications for its use in treatment, as well as the precise indications for rest, occupation, and diet.

The possibilities of protective vaccination and of chemical therapy.

The significance of mixed infections, and the factors concerned in toxemia.

The effect of the antituberculosis campaign, and the relative importance of its various phases.

The difference in duration of life between the patients who follow our directions and those who do not.

As already stated, the list does not pretend to be exhaustive. It is rather illustrative of the little we know and the much we need to learn in order to act with confidence in the situations we have to meet. Our practical object is to reduce tuberculosis, our dream to eliminate it. Are our methods rational? Does our effort to keep the sick alive help or hinder the main achievement? Is it desirable to prevent exposure, or do we thus develop a vulnerable race, a race untrained in defense? These are questions which we cannot answer. All we can say is this: We may reason for the race and long for omniscience, but we must act from more immediate motives, and to the best of our present ability. We must use the little wisdom and skill we have to relieve and protect those who depend upon us here and now.

—*G. B. W. and C. T. R.*

The Control of Measles

BROWNLEE[1] recognizes the great difficulty in controlling or limiting the spread of measles and makes some suggestions which are of value. He states that among the middle classes in England it is observed that children rarely take this disease until the eldest in the family reaches the school age. This is not so markedly the case with the working classes. Among these, greater facilities of infection are afforded by the children playing together in the streets. In spite of this, however, in the opinion of a number of medical officers, the fire is lighted in the schools and the flame is carried to the streets. Under six months of age measles is not likely to be acquired, but when acquired the death rate is fairly high. Brownlee finds that most cases occur between the ages of six months and six or seven years. If it be assumed that in families of young children the children do not as a rule take measles until the eldest child has become sufficiently old to go to school, something can be done to prevent its spread, and this is suggested by Brownlee as follows:

[1]British Medical Journal, April 17, 1920.

"Each school will keep a register. When a child of five years is admitted to a class at school it will be noted in the record whether that child is the eldest, intermediate, or youngest member of the family. When measles breaks out in a class the register will be consulted. If the child exposed to infection be the youngest member of the family, nature may be left to take her way—there is no further danger. But if he or she be the eldest, especially if the younger children in the house are of ages between six months and three years, the direct action of nature is no longer a matter of indifference. What is a mild disease at the age of five years may be a matter of grave danger at the age of six months. Now it may be taken as practically certain that very few children develop the first symptoms of measles within seven days of the infection, and the higher limit may be set at fourteen days. If, therefore, the child who has been exposed to the infection stays in its home for seven days after exposure no harm will ensue. It is the next seven days which are important. In the entourage of families in towns, especially where the families are very young, the house of one grandmother is usually available. There are also quite frequently houses of uncles and aunts in which there are either no children or in which the children have already passed through the necessary attack of measles. It is thus only a matter of arrangement that the child who has been exposed to infection stays with a grandmother or other relative for a specified seven days. It may be objected that this cannot be done. On the contrary, I have made such arrangements for many years with reference to cases of scarlet fever and diphtheria. Where on dismissal there was some doubt as to whether a patient were free from infection or not, and where it was obvious that continued residence in the hospital was not the best way to clear the patient of infection, I at once appealed to the parents. I found that arrangements made were loyally carried out in nearly every case; the average parents are not selfish as regards their children, but much the reverse. There are, of course, a number of unreasonable and untrustworthy people, but in my dealings I have found these the exception. Once the matter has been carefully explained the ordinary person wishes to act for the best. Of course, at the institution of a new method of administration there will be a considerable amount of evasion, but from the moment that the opponents of the system see their children going to the grave while those who accede find their children do not take the disease, the sarcasm of the neighbors will affect more than thousands of regulations. A few years' trial will put all the community on your side, and then the work is done."

Brownlee emphasizes the fact that early diagnosis is a matter of prime importance in controlling measles. He states that there is no reason for believing that this disease is transmissible until the appearance of the first catarrhal symptoms. For early recognition purposes there are only two symptoms and signs that are of value. The usual advice is to look for Koplik spots, but by the time these are in evidence the disease has in all probability been transmitted. The temperature is one of the early evidences of this disease. When a child comes to school with suffused eyes and edema of the lower lids and is found to have a temperature, it may or may not be developing the measles, but it should be immediately isolated under the best conditions possible and kept in bed for at least four days. If it be measles, at the end of that time the eruption will appear.

Incidentally, Brownlee makes it evident that the case mortality in this disease is much greater among those treated in hospitals than among those treated in their homes. He gives figures for two cities, Aberdeen and Glasgow. In the former, most children with this disease are treated in their homes. In Glasgow, on the other hand, Brownlee's statistics include only those treated in hospitals. The case mortality in Glasgow is much higher at all ages than in Aberdeen. It may be that there is some other factor besides hospital treatment accounting for the difference in the death rates in the two cities, but this is the testimony nearly everywhere. Uncomplicated measles is a mild disease and has a low death rate, but the virus of this disease opens gateways to secondary infections, and these kill. Whether a child with measles will be more likely to escape secondary infection in its home or in a hospital depends upon conditions which must be judged by the medical man in charge. In a contagious disease hospital constructed and manned in a modern way the child with measles should find its safest place, but many such hospitals do not come up to this standard. The virus of measles is carried through the air for short distances, but in hospitals secondary infections are usually transported by careless attendants. The nurse in a measles hospital in going from one patient to another should exercise the same care and practice the same precaution as would be done if one of these children had measles and the other had scarlet fever. One child may harbor a deadly streptococcus and the careless attendant may carry this organism to every other child under her charge. Until the importance of preventing secondary infections in measles is understood and efficient methods of prevention are practiced the child with measles, under ordinary conditions, is much safer in an isolated room at home than it is in a hospital ward.

—*V. C. V.*